

nature

October 2, 1975

Dear Sir...

Thank you for your application to the Astronomical Research Council for a grant to support the project "Determination of past sunspot numbers from mediaeval records of the swede harvest". The council unfortunately felt that this work was predominantly within the domain of the Vegetable Research Council to whom you are recommended to apply.

Thank you for your application to the Vegetable Research Council for a grant to support the project "A study of the past swede harvests and extraterrestrial influences on them". Unfortunately, the council felt that this work was too interdisciplinary to come within its purview; it recommends you to try the Interdisciplinary Research Committee.

Thank you for your application to the Interdisciplinary Research Committee for a grant to support the project "Stellar magnetohydrodynamics, solar disturbances, atmospheric impacts and their meteorological consequences for agricultural productivity based on mediaeval records". Although the committee felt the research was timely, it feared it did not have enough promise in it. But have you tried one of the foundations—they can often support this sort of work?

Thank you for your application to the Doogood Foundation for a grant to support the project "Man's well-being improved by a better understanding of the influences on agricultural productivity". The foundation felt that the work, although showing great promise, could not yet be regarded as timely. Maybe there are some funds available in your own university.

Thank you for your application to the Miss Hygienia Philpott Fund, for support for your project "A search through mediaeval records within the university". It is true that Miss Philpott's Will did encourage research within the university archives but she added the rider "insofar as this may tend to increase the virtue and godliness of the scholars of the university". The trustees of her Will regrettably could not see such an outcome in this case; surely a government department would support such work?

Thank you for your application to the Ministry of Commerce for support for your project "Improving the balance of payments by a more profound knowledge of the forward market in swedes". We have several laboratories employing thousands of scientists; the project would be ideal to give them something to do, but we couldn't give support for an outsider to do it. Couldn't industry help?

Thank you for your application to the Giant Swede Corporation for support for your project "Better swedes

within the next eleven years". Unfortunately the people in our research laboratory are not too keen on ideas that they have not initiated themselves. Furthermore I should add that the amount of money the swede industry has available for research is very small. Might I recommend you try an organisation with more funds?

Thank you for your application to the Ministry of Defence to support the project "Historical variability in the swede harvest and problems of feeding armies; possible causes". Although it is true that we have substantial funds available for research, we are under growing pressure to demonstrate that this research is relevant to modern military methods. Might I suggest you reword your application appropriately and send it to the Ministry of Attack?

Thank you for your application to the Ministry of Attack to support a project "More effective thermonuclear weapons through better understanding of time-dependent thermonuclear processes in the Sun". Unfortunately the Minister is increasingly worried by the damage that students are causing to university laboratories at which military research is being done. Maybe you could disguise your work with a humanitarian purpose.

Thank you for your application to the Cancer Foundation to support a project "Do sunspots cause cancer directly or through swede harvest variability?" I'm afraid we found the proposal a little far-fetched. Maybe a private benefactor would help.

Thank you for your letter to the Twelfth Marquess of Cumberland requesting support for your project "Harvests were better in the olden times". Unfortunately His Grace is a trifle penurious these days what with swingeing taxation, and he writes from Bermuda to recommend that you follow his example of collecting money in small quantities from large numbers of people in return for some favour that you can bestow upon them.

It has been brought to the attention of the Director of Public Prosecutions that you together with some students were to be seen last Saturday dressed as swedes and offering the public a look through a telescope at the Sun in return for 10p to "support research". Since I do not find your name on the list of registered charities licensed to collect in the streets, I must warn you that any repetition of this offence will lead to prosecution. Besides, there are perfectly normal channels for obtaining money for research purposes; perhaps you are unaware of the government's handbook which lists them (alphabetically) as the Astronomical Research Council, . . .

... Your obedient servant





The first fuel bundle at the Duane Arnold Energy Center is lowered into its slot in the nuclear reactor, February, 1974.



Nader: anti-nuclear leader.

Energy policy in the United States assumes that there will be a marked expansion of the nuclear power programme, with as many as 1,000 nuclear power stations in operation by the year 2000. But the nuclear industry is now experiencing some of the toughest political and economic challenges it has ever faced. In the first of three articles on U.S. nuclear policy, Colin Norman reports from Washington.

IN his first major statement on energy policy, President Ford last January declared that he firmly supports a massive expansion of the nuclear energy programme in the United States. Without such an expansion, he argued, there is little hope that the nation can free itself from its painful dependence on foreign sources of oil, and he announced that his Administration would strive to ensure that at least 200 nuclear power plants are generating electricity by 1985. That would represent almost a fourfold expansion, from the present 55 nuclear stations.

With such a strong verbal commitment from the President, backed by more than \$1,000 million in the present federal budget for a variety of nuclear energy programmes, it might be supposed that the nuclear industry is in the midst of a bonanza. But it isn't. Instead, it is under siege, and it is facing a very uncertain future.

It is caught up in a complex web of technical, regulatory and economic problems, under assault by articulate and resourceful critics, facing increasing scrutiny by the Congress, and unsure of its public support. In fact, the problems are so serious that earlier this year a government task force concluded that "the nation's nuclear power program is approaching a state of crisis", and it added that it "is not optimistic that in the restrictive environment in which the nuclear industry operates today . . . actions can be implemented quickly and completely enough to avoid significant restriction on the growth of fission energy use". The task force which made those statements consisted of some of the top officials in charge of the federal government's nuclear energy programme.

One of the most acute problems afflicting the nuclear industry is an economic one—the national economic recession has more than made its mark on the industry. New orders for reactors, which not very long ago were coming in thick and fast from electric utility companies, have slumped recently, and the utilities have also been forced to postpone or cancel scores of orders which they placed before the recession began to bite. The figures speak for themselves: in the 18 months which ended on June 30, firm orders were placed for only 34 reactors, 17 reactors ordered previously were cancelled, and construction of 167 others was postponed for periods ranging from six months to indefinitely.

On top of those acute—and possibly short term—economic woes, the industry finds itself operating in a new and, its supporters insist, more hostile political environment in Washington. The demise earlier this year of the Atomic Energy Commission (AEC) and its replacement by the Energy

Research and Development Administration (ERDA) demolished the bureaucratic structure which nurtured the early growth of the nuclear power programme, leaving the industry devoid of a solid power base in the federal government to look after its interests. Nuclear energy is now just one of five divisions in ERDA, and it must compete directly with other technologies for its share of the energy research and development pie.

And the industry's political base in the Congress is also fast being eroded. Congress is just beginning to take a searching look at the nuclear power programme, and for the first time in nearly 30 years, the powerful Joint Committee on Atomic Energy, which has jealously guarded the interests of the nuclear programme, has been forced to yield some of its monopoly over atomic energy affairs. A subcommittee of the House Interior Committee, under the chairmanship of Representative Morris Udall—a declared candidate for the Presidency—has been conducting prolonged and well publicised hearings on some of the more controversial aspects of the nuclear power programme, and the Congressional Joint Economic Committee has also been delving into the tricky questions of nuclear energy finances. Moreover, the Joint Committee on Atomic Energy is itself an entirely different animal from what it was just a year ago; two of its longest serving, and most powerful, members have left Congress, and the committee has benefited from an influx of more sceptical members of Congress.

This altered political environment sprung up just as a number of controversial issues, which will fundamentally affect the future growth of the nuclear industry, are coming to a head at the national level. In the next few years, the federal government will have to decide the structure of the uranium enrichment industry, how to dispose of radioactive waste material, whether to allow plutonium produced by reactors to be recycled as reactor fuel, and whether a new generation of breeder reactors should be built.

But, although those issues affect the long term structure of the industry, a more direct challenge to present-day nuclear operations is beginning to emerge at the state and local level. For the past four or five years, applications to build nuclear power plants have been challenged by local environmentalist and anti-nuclear groups, intent chiefly on keeping nuclear power plants out of their neighbourhoods. The groups have operated, more or less in isolation, by fighting the nuclear industry on a case-by-case basis. But recently their activities have become more coordinated, and a loose coalition has formed with

with a charismatic and highly effective leader—Ralph Nader.

The political effect of the local anti-nuclear groups is already being felt. According to a survey carried out in May this year for the Atomic Industrial Forum (AIF), the nuclear industry's trade association, there are bills pending in virtually every state legislature in the country which would to some extent curb the growth of nuclear power at the local level. Though very few of the more restrictive measures stand any chance of being passed, the sheer volume of the legislation is causing some concern among nuclear advocates. But a new development is causing even more concern.

Earlier this year, a coalition of environmentalist groups in California gathered together more than the 313,000 signatures needed to call a state-wide referendum on nuclear power next year. The referendum, in the form of a proposition which will be placed on the ballot papers for the Presidential primary elections in June, is a cleverly worded resolution which would forbid nuclear plants from being built in California unless they meet stringent safety criteria, unless safe waste-disposal measures have been devised, and unless the public is fully insured against the consequences of nuclear accidents. Moreover, if existing power plants in California don't meet the standards (which at present they don't), the proposition states that they would have to be gradually phased out of operation over a period of several years.

Ed Koupal, a Californian who organised the petition, is quick to point out that the measure "is not against nuclear power, it is for nuclear safety", but the net effect of the proposition—if it is passed—would clearly be to impose a moratorium on nuclear power in California. Though its chances of being passed are generally considered to be slim, there are two strong factors working in its favour. First, the concept embodied in the proposition—that nuclear power should only be allowed if it can be shown to be safe—is easy to sell in an election campaign. And second, a new California state law drastically limits the length of a campaign and the amount of money that can be spent in a state-wide election, which means that the nuclear industry cannot launch a massive propaganda blitz to get the measure defeated.

California isn't the only state likely to hold a referendum on nuclear energy matters next year. A group of environmentalist organisations, loosely coordinated into a coalition called the Western Block, is trying to get resolutions similar to the California proposition placed on the ballot papers in the November Presidential elections in 16

other states.

In short, therefore, the nuclear energy industry finds itself facing some of the toughest political and economic problems it has encountered in its troubled history, while, at the same time, the conventional wisdom among energy planners in the federal government is that a massive expansion of the nuclear programme will be vital to meet growing energy demand.

The industry is gearing up to put its case across to Congress and the general public. The AIF, for example, is stepping up its public relations campaign; it has moved its headquarters from New York to Washington, doubled its budget to \$1.2 million a year, and is already sending out reams of paperwork to the news media. AIF officials say that they do not plan to do any direct lobbying because another industrial organisation has been set up for that purpose. Called the Nuclear Energy Council, it is headed by former Representative Craig Hosmer, who was the ranking Republican on the Joint Committee on Atomic Energy until he retired from Congress last year.

In several respects, the industry has a number of advantages over its critics in its bid for national attention. Not only is the AIF able to outspend the nuclear critics by almost an order of magnitude, but it is much better organised and has far greater resources to draw on. Moreover, it recently received a considerable boost from a public opinion poll, carried out by Louis Harris. The poll revealed that 69% of the public would approve an expansion of the nuclear power programme in the United States, while only 19% of the respondents declared themselves opposed to such a move.

The chief nuclear critics operating at the national level are Ralph Nader's organisation, the Union of Concerned Scientists (UCS) and the Natural Resources Defense Council (NRDC). The UCS has provided most of the technical backing for challenges to the safety of nuclear power plants, and its principal leaders are Henry Kendall, an MIT nuclear physicist, and Dan Ford, an economics graduate. The NRDC has been most active in attacking health standards associated with plutonium, safeguards against the theft of a weapons-grade nuclear material, and the government's arguments in support of the liquid metal fast breeder reactor programme (LMFBR). Tom Cochran, a nuclear physicist, Arthur Tamplin, a former AEC health physicist, and Gus Speth, a lawyer, form the basis of NRDC's nuclear attack force.

The debate on the desirability of nuclear power has, of course, been going on for several years, but its focus has shifted considerably. Early con-

cern about the health effects of the releases of small amounts of radioactivity from power plants, and worries about the environmental effects of thermal emissions, have greatly diminished—chiefly because the standards have been considerably tightened. The most controversial issues now are the safety of light water reactors, hazards associated with plutonium, the proliferation of nuclear weapons, and the difficulty of finding a safe method for disposing of highly radioactive reactor wastes. Those issues will ultimately have to be resolved by Congress.

Congress will have to make up its collective mind on the following matters.

Energy demand. An underlying feature of the entire nuclear energy debate is a fundamental divergence of opinion on energy policy. The nuclear critics, in short, argue that if stringent, mandatory energy conservation measures are applied, and if research and development on technologies for using solar energy are stepped up, the nuclear programme can be slowed down and there will be no need to develop the breeder reactor. The most forceful backing for that viewpoint was incorporated into the findings last year of the Ford Foundation's Energy Policy Project, a massive analysis of energy options which cost \$3 million to complete. But the Congress has so far shown no stomach for taking the unpalatable political decisions needed to slow down energy growth, and federal energy policy is firmly based on the assumption that, although energy growth may be slowed a little, the growth in demand for electrical energy will continue to be large. In the past few years, consumption of electrical energy has been increasing by about 6% a year. Consequently, in order to stave off increased oil imports, new generating capacity will have to be provided by coal and nuclear fission.

Those assumptions are firmly embodied in a long term energy research and development plan published on June 30 by ERDA, which will form the blueprint for ERDA's operations over the next few years.

Reactor safety. In the past few years, the central issue in the nuclear power debate has been whether or not the many safety features built into light water reactors can reliably guard against a major accident which would release large quantities of radioactivity into the environment. The focus of the debate has been an arcane piece of plumbing known as the Emergency Core Cooling System (ECCS), which is designed to flood the reactor core with water if a pipe breaks and the reactor loses its main supply of coolant.

The issue of the reliability of the

ECCS was first brought to public attention by Kendall and Ford of the UCS, who successfully badgered the Atomic Energy Commission into holding prolonged hearings on the matter in 1972 and 1973. Though the hearings failed to resolve the dispute, the AEC tightened its operating standards for ECCS in December 1973, and it is planning a full scale test of the device in an experimental reactor in Idaho in the late 1970s. The critics have not been slow in pointing out that scores of reactors are already being built with an essentially untested safety device.

Some of the steam has, however, been taken out of the safety debate by a massive analysis of the probability of a catastrophic reactor accident, which was carried out for the Atomic Energy Commission by Professor Norman Rasmussen of MIT. A draft of the report, published last year, offered the widely quoted conclusion that the probability of a severe accident resulting in the release of large quantities of radioactivity, is extremely small, that it would result in about 2,300 deaths, and that it would cause about \$6,000 million of damage to property. The report has stimulated a good deal of controversy, however, and some of its findings have been challenged by a separate analysis by the American Physical Society.

In the meantime, however, a fire which occurred in March at the Browns Ferry nuclear power station in Alabama—the world's largest nuclear power plant—reopened the safety dispute. Caused by an electrician checking for air leaks with a candle, the fire knocked out the ECCS system at the plant, and raised a number of questions about the ability of plant operators to react to an emergency.

Plutonium and the LMFBR. Most of the present controversy surrounding the nuclear power programme is concerned with the possibility that plutonium, produced in nuclear reactors, may be recycled and mixed with uranium as reactor fuel. Worries about the toxicity of plutonium have arisen, chiefly in connection with a theory put forward by Cochran and Tamplin of the NRDC, that tiny inhaled particle of plutonium could lodge in the lungs, providing the surrounding tissues with a massive dose of radioactivity. Though the theory has been hotly challenged by various scientists, it is still giving rise to considerable debate. But a more prominent and worrying problem with plutonium is the possibility that quantities of it could be stolen from the nuclear industry and fashioned into crude nuclear bombs, either by terrorist groups or by countries which possess nuclear reactors, but which have not yet produced nuclear weapons. India's development of a nuclear explosive

from plutonium derived from a reactor supplied by Canada has brought the problem to public attention rather dramatically.

Because of uncertainties surrounding the adequacy of safeguards to prevent illicit diversion of plutonium from the nuclear industry, the Nuclear Regulatory Commission (NRC), which has assumed the regulatory functions of the AEC, announced earlier this year that it will probably defer making a decision on whether or not to allow plutonium to be used as a reactor fuel until 1978.

The nuclear industry is upset by the NRC's delay because, it argues, plutonium recycling will be needed to supplement dwindling supplies of uranium; it would reduce requirements for enriched uranium by 10% in the early 1980s. Congress may well have to decide the matter eventually, since a bill outlawing plutonium recycling until the uncertainties have been resolved has been introduced into both the House and the Senate.

Plutonium recycling is itself, however, only a prelude to introduction of the breeder reactor, which is designed to produce more plutonium than it consumes as fuel. The breeder reactor programme is the single most costly energy research and development project supported by the federal government, but its advantage is that, whereas light water reactors would run out of uranium fuel in a few decades, the breeder could provide power from fission for several centuries.

Major disagreements have erupted over the timing of the programme, its cost—the research and development effort alone is expected to swallow up about \$10,700 million, compared with an estimate just three years ago of \$3,300 million—and whether or not other, more acceptable, technologies can be developed in time to make the breeder redundant. A major challenge was offered to the budget for the breeder programme in Congress this year, but it was defeated.

Waste disposal. A major uncertainty afflicting the nuclear programme at present is what will eventually be done with the highly radioactive waste material produced in the reactor core. Although it is generally expected that burying the wastes deep in a salt mine will eventually prove to be safe and acceptable, the concept has yet to be thoroughly tested, and a suitable site has yet to be found. A site was prepared for testing in Kansas in the late 1960s, but it was found to be punctured by abandoned oil and gas wells; the project ran into spirited local political opposition and it had to be scrapped. Consequently, the Atomic Energy Commission announced that it would store radioactive wastes above

FUNDS to support research are becoming scarcer, so it is increasingly important to ensure that the money available is used as profitably as possible. For some years I have been suggesting that we are getting poorer and poorer value for the public money voted for research, and that an increasing fraction is being wasted on unnecessary and complicated administration. One of the fears aroused by the Rothschild recommendations was that they would make for additional complications and further waste. Even the most enthusiastic supporters of these changes cannot deny that there are many more scientists, individuals who were previously themselves engaged in research at the bench, who are now desk-bound planners, and that research funds are also spent on many more non-scientific administrators supporting (or even controlling) the scientific administrators. The hope is that this will result in the remaining bench workers devoting their time more profitably to more important projects.

In the meantime, it may be useful to try to find what sort of organisation is producing the most for the least expenditure. In the past, I have been accused of sentimentality for looking back nostalgically at the small units which operated efficiently in the 1930s, when funds were even tighter than today. I have been told that it is impossible to "put the clock back". The assumption is that the age of string, sealing wax and enthusiasm has gone for ever.

I have therefore been looking with interest at the performance of the Oil Pollution Research Unit of the Field Studies Council. Here we have a small group of young scientists working in converted cellars and outbuildings on a smaller total budget than that enjoyed by many individual academics who have received research council grants for studies which have made little noticeable impact on science or human welfare. At an international conference held at Aviemore in Scotland in April of this year, and sponsored by the Institute of Petroleum and the Field Studies Council, members of the unit described their work. It was clear that this was both important and entirely relevant to the needs of the petroleum industry and to understanding the likely ecological effects of the exploitation of oil reserves. The work fulfilled all the

Lab charges



KENNETH MELLANBY

requirements that the Rothschild proposals are intended to produce.

The Oil Pollution Research Unit (OPRU) has, however, shown that a very high proportion—more than 90% I would estimate—of their funds are actually spent directly on research, paying salaries, equipping laboratories, hiring facilities and visiting sites. The parent body, the Field Studies Council, is itself an admirable organisation, with the least top-heavy administration of any comparable body. They do not have time to interfere in the running of the OPRU, though they are available to give help where this is required. The main lesson to be learned from this research organisation is, once more, that "small is beautiful", or at least that the small unit can still be the least wasteful.

I think that many other organisations could learn from this example. Their overheads are very much higher, though their productivity is, on the whole, lower. We hear many complaints from government departments, who have funds to commission research, that some laboratories run by research councils, or even by other government departments, are in danger of pricing themselves out of the market. This is because most contracts must bear a surcharge of 100% to meet overheads and administrative costs. It is difficult not to suggest that these could be greatly reduced by a simpler administrative structure.

There is even some criticism of university departments, which are

accepting contracts and making a much smaller surcharge. This is, in some circles, considered to be unfair competition. It is suggested that they should be compelled to charge more, for they are accused of not costing their work properly. As one with some experience of university administration, I do not think that this is the case, though the argument might appeal to empire-building registrars wishing to increase their own staff numbers. If I, as head of a department, had accepted a research contract, and it had included all the costs of staff and their equipment and materials, I would not have spent a penny more on running the department, nor would there have been any need for the central university administration to increase its costs. I might have had to write an occasional letter, but I would have hoped to compensate for this, and more, by persuading the research workers to give a few of my lectures on subjects in which they possessed a particular expertise. I would have looked upon my relationship with the contract team as one of symbiosis, and not treated them as parasites.

My main criticism of many university and polytechnic staff today is that they often assume that they cannot do worthwhile research unless they can land a substantial grant for that purpose. Even junior lecturers think they are justified in asking for a graduate research assistant. Yet even when they obtain this extra money, many are obviously less productive than the pre-war lecturer who was lucky if he could obtain a few pounds from departmental funds for extra materials. It may well be that the present shortage of money will have its good results. Research workers may have to eschew problems requiring costly apparatus (much of which is accumulating dust up and down the country) and devote more time to thinking how to tackle problems within their own resources. They may even realise that some of the large grants which have been floating about in recent years have actually been counter-productive. We are said to be facing, in Britain, a financial crisis even more serious than that experienced in the 1930s. We should therefore remember that, in many subjects, it was then that Britain led the world in scientific research. □

the ground in a temporary facility until an ultimate waste disposal method has been found. Earlier this year, however, ERDA announced that it was reconsidering the whole problem and would issue a statement on the matter in due course.

With those disputes unresolved, and

reactor sales at a virtual standstill, the immediate prospects for President Ford's goal of bringing 200 nuclear reactors on line by 1985 looks slim. But, in the absence of a strong, coherent energy policy featuring mandatory energy conservation and massive support for non-nuclear energy

technologies, the nuclear power programme represents the only proven alternative to greatly increased oil imports. Faced with that prospect—Congress will be forced soon to take some controversial decisions. In the meantime, the nuclear energy industry will have to live with uncertainty. □

international news

At the opening last week of the annual conference of the International Atomic Energy Agency in Vienna, the Director General, Mr Sigvard Eklund, expressed confidence in the continuing expansion of nuclear power technology. He suggested that nuclear energy based on fission provided the only immediately available alternative to fossil fuels in spite of recent optimism about the potential of alternative energy sources.

Addressing the delegates of 107 member nations of the IAEA Mr Eklund said that as alternative resources from solar, geothermal and wind power, and from nuclear fusion would not become readily available for some time and that even then those sources would provide less power than current estimates suggested.

Mr Eklund was, however, optimistic that largely under the guidance of the IAEA the problems associated with the use of fission reactors could be overcome. These included three main issues which have recently formed the focus of public debate, namely, the safety and reliability of the reactors, the disposal of radioactive waste, and the need to ensure that the spread of nuclear technology does not lead to a proliferation of nuclear arms development among the non-nuclear powers.

Dealing with the first of these points Mr Eklund said that the IAEA is engaged on a programme to formulate a comprehensive system of international

IAEA head puts faith in nuclear power

by Allan Piper

safety codes and guidelines for nuclear power plants. The agency believes, he said, that the "teething troubles" undergone by the larger reactors have now been eliminated, and that it is no longer fair to regard nuclear power stations as unreliable.

Many of the problems associated with the satisfactory management of waste products remain to be resolved, however. In particular, the IAEA is establishing a standing advisory group to examine the feasibility of burying waste in geologically stable terrain. Referring to that concept Mr Eklund drew attention to the point raised during an earlier agency conference that plutonium formed in the Oklo Formation about 1,700 million years ago decayed quite harmlessly without escaping into the environment.

On the question of preventing an escalation of nuclear weapons proliferation as more countries obtain the materials for developing nuclear power plant Mr Eklund stressed the need for the adoption of internationally accept-

able IAEA safeguards. He expressed disappointment that though the Non-Proliferation Treaty Conference held in Geneva in May expressed strong support for safeguards controlled by IAEA their recommendations had not been considerably more decisive.

Calling upon the present nuclear powers as the major exporters of nuclear materials and technology, Mr Eklund urged them to take the lead by supplying nuclear resources only to those nations prepared to place their "entire nuclear activity" under international safeguards.

He stressed that commercial and political interests should not prevail over that objective, adding that further progress towards a comprehensive ban on nuclear tests by the nuclear powers themselves would assist by setting an example to the developing countries. He also proposed that the explosion of nuclear devices necessary to the development of nuclear technology be placed under international control.

Though Mr Eklund's speech reflects a confidence in the future of nuclear fission as a major source of energy he indicated that IAEA are not discounting altogether other possible sources. The agency, together with the World Health Organisation, is, he said, co-operating with the International Institute of Applied Systems Analysis on a programme to evaluate the viability of every available option. □

M. d'ORNANO, the French Minister for Industry, has presented the conclusions of the consultative commission on energy, chaired by M. B. Gregory, the Director General of the CNRS (National Centre for Scientific Research). This commission was set up to examine the future direction of government energy policy, and includes representatives from a very wide spectrum of interests—specialists, energy users, and "social partners"—so that all shades of opinion can be expressed. Three criteria were drawn up to help the working of the commission—low financial cost, political independence, and ecological and social aspects, but the commission "regretted that it could not report within this framework any coherence in the proposed courses of action". In fact, the commission's report is not always in agreement with decisions reached by the government.

In its plans for 1985, the commission

places special emphasis on efforts to reduce energy consumption. By comparison with 1973 forecasts, the total consumption hoped for would be 240 Mtpc (millions of tons petroleum equivalent) instead of 285 Mtpc, and the amount derived from petroleum products would be 96 Mtpc instead of 178 Mtpc. The report stresses that "this is compatible with an annual growth rate of 5.5% only at the price of a policy involving strict restraint, but it is well worth the effort to attempt to reach this goal". The feasibility of the 5.5% rate as the basis of a working hypothesis has, in any case, been thrown into doubt by some members of the commission. Coal consumption (30 Mtpc) would continue to decline progressively, while gas consumption would rapidly increase to 37 Mtpc.

The recommendations of the commission regarding electricity are very

precise: whereas electricity production would, at the present rate, have approximately doubled by 1985, the commission underlines the dangers of a policy exclusively favouring electricity; it urges that major research and development efforts be made on solar, hydrogen and other energy sources.

The most striking point of the report is without doubt the split which has appeared within the commission over the nuclear programme. Some of its members advocate, for safety reasons, the construction of nuclear power stations of lower output than that proposed by the government, the balance of the energy being provided by traditional power stations even if this seems less favourably economical. M. d'Ornano has perhaps shown excessive optimism in making the report of the Gregory commission public, for it seems to call into question many aspects of government policy. □

France boosts nuclear physics

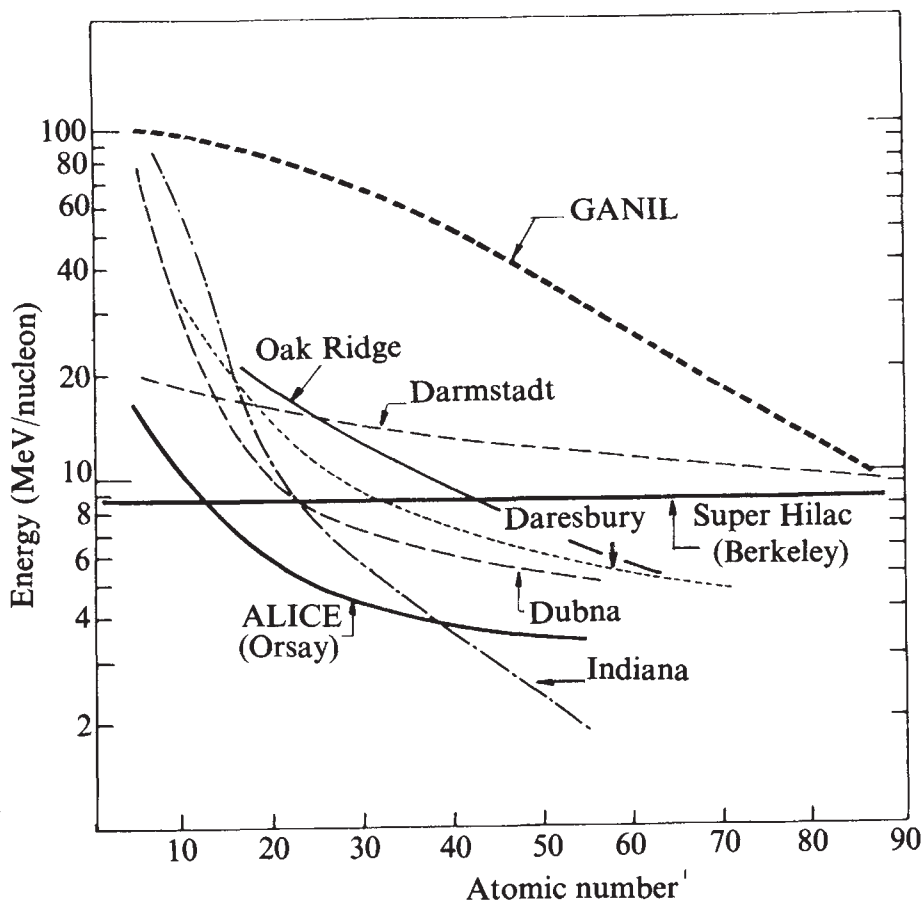
by Roger Woodham

A NEW experimental facility for 'low energy' nuclear physicists, to be known as the Grand Accélérateur National à Ions Lourds (GANIL), will be included in the French Seventh Plan which covers the years 1976-80. It will cost about £20 million, should be in operation before 1980 and is to be built just outside Caen in Normandy.

As well as being part of the French government's strategy for livening up the French economy by authorising spending on new scientific projects during the next five years, the Caen decision also has implications for the recently acquired French interest in regional policy. With the mood in France at present much in favour of decentralisation, the Délégation Générale à la Recherche Scientifique et Technique (DGRST) has taken the first tentative step towards some sort of regional policy in science by giving the green light to GANIL.

Instead of choosing to build the new accelerator complex in the vicinity of Paris, perhaps at Saclay or Orsay, the DGRST and the Minister of Industry and Research, M. Michel d'Ornano, settled on Caen—a somewhat surprising selection which was not on the list of places like Grenoble, Strasbourg, Lyon and Bordeaux which have been widely canvassed during the past year or so. Cynics like to point out that M d'Ornano is Mayor of Deauville (not far from Caen) and was until last year President of the Regional Council of Lower Normandy. The fact is, however, that Caen is only two hours by a fast train service from Paris and will therefore be easily accessible to nuclear physicists from other countries, a significant consideration given that the French see GANIL as an important international facility.

The GANIL project, which will make available beams of heavy ions ranging from carbon to uranium at energies higher than is or will be possible with machines elsewhere, is the brainchild of a coordinating committee set up by the DGRST in 1972 to look at the whole matter of French support for nuclear and elementary particle physics. (In France these activities come under the auspices of the Commissariat à l'Energie Atomique (CEA) and the Institut National de Physique Nucléaire and et de Physique des Particules, the latter being an umbrella organisation colloquially known as IN2P3 and covering all nuclear and particle physics activity in university laboratories and those of the CNRS (Centre National de la Recherche Scientifique).) GANIL was



A comparison of the energy per nucleon available from GANIL (planned) compared with that available (or planned) at other accelerators.

dreamt up by a working party drawn from the CEA and IN2P3, and the money required will be provided in equal measure by the two organisations.

There are two somewhat unusual features of GANIL: first, that the main part of the system is to comprise two large cyclotrons, one of which will inject particles into the other, and second that superconducting magnets, which are increasingly in evidence in designs for new accelerators, are not to be used. As to the cyclotrons, the GANIL team maintains that it is only by using this kind of accelerator that high values of the energy per nucleon of a nucleus can be achieved. Certainly, if the promise implied in the figure is upheld, the French will have something to be proud of: over much of the range of atomic numbers the energy per nucleon available from GANIL is several times higher than from other accelerators.

The GANIL team decided against superconducting magnets mainly on the grounds that France does not have enough experience to take what might amount to a very risky step, especially as others have hit snags while building such magnets for accelerators. The team argues, however, that a cyclotron system is absolutely necessary and is

to be preferred to the tandem Van de Graaff arrangements to be used at Daresbury in the UK, for example. Certainly the linear machines at Darmstadt and Berkeley, for example, are capable of accelerating greater numbers of particles per second, and the quality of the beam from a linear machine is on the whole better than from a cyclotron, but when it comes to increasing the energy delivered, the cyclotron seems to win hands down.

Not all users of GANIL will have to avail themselves of the maximum energy available, however. Many of the people who will eventually use the facility are chemists, biologists and so on, and it will often be that they will use beams of particles from the first cyclotron, before they are injected into the second one for further acceleration. In any event, the site for GANIL—which is still a green field on which no more than preliminary geological investigations have been made—is close to the Faculty of Medicine of the University of Caen, and to a college of technology; there is also ample room for interested industrial concerns like electronics companies to set up their own bases nearby so that they can make use of GANIL for their own investigations, on ion implantation, for example. □

ONE of the more practical ideas to come out of the Stockholm Conference on the Human Environment in 1972 was that of setting up some sort of central service for the collection and dissemination of information on a very wide range of environmental matters. Before the conference, some people seem to have envisaged a centralised service actually providing information, or at least providing direct references to precisely where it could be obtained. But a more realistic look at the situation, and in particular an appreciation of the wide range of subjects and problems to be covered, indicated that this would not be feasible, at any rate on a global scale. Even if a centralised system had been a practical proposition, it would be quite impossible to keep information up to date, especially with environmental affairs in their present unstable and rapidly developing state.

In fact, what the conference organisers had had in mind all along was an obvious alternative, in the shape of a referral service. This would not itself provide or store information, but would aim to put its clients in touch with those places where the information they seek could be found, and to do this in an orderly, internationally accepted fashion. After two years of careful planning and consultations with governments, research institutes and other organisations with relevant experience, the International Referral Service (IRS) has recently started to operate. In view of the wide field to be covered, the UN Environmental Programme (UNEP) decided to base the service on a number of priorities considered by members of its Governing Council to be of global importance. Because the service must also be useful within individual countries, however, or to clients having purely local interests, it needs to encompass a wider definition of the general concept of "environment" than is generally understood by the UNEP itself in its day-to-day working.

A service that does not itself provide information, but is designed to indicate where that information can be found, will obviously have smaller storage requirements than a centralised direct information service, and will not need such extensive updating facilities; nonetheless, more is needed than the UNEP can provide, although it is not in fact necessary even for the basic information to be centralised. As now set up, therefore, the IRS works through a number of 'focal points', notionally one for each contributing country. A typical focal point has two roles: first, it acts as a centre for the collection of information and, second, it functions as a subsidiary of the IRS within its own country. It will thus be able to

operate in two ways:

- By collecting and storing detailed information not required by the IRS but needed locally.
- As part of the overall IRS network. Operation at the purely local level recognises the fact that most countries now either have, or intend to have in the near future, some sort of environmental control mechanism, which will, however, inevitably trespass on areas already covered by other government departments. The sets of information available within countries are in fact largely in the areas so covered, and

UN environmental service launched

from Peter Collins, Geneva

the national environment organisations are thus best suited to cataloguing sources of such information within their own countries.

Being financed by government and therefore liable to government control, a national focal point also controls itself as regards its clientele. It is expected to evaluate its own sources and to keep out trivial requests, thus giving the system a good deal of flexibility and national control. The only clients other than those approved in this way by the focal points are the members of the UN system, who could be among the biggest users of the new service, and non-governmental organisations having official status. At the other end of the chain, the national focal point can also decide whether or not to accept a request, although it is not anticipated that this sanction will often be applied: a request already passed by the focal point in its country of origin is unlikely to be rejected. The answer to a request goes back through the same channels as those through which it was received.

From the practical point of view, the essential document is the IRS Operations Manual, the international version of which appeared in August this year. This comprises clear and concise guides to the various elements of the service—sources, focal points, and users, giving the purpose and responsibilities of the first two and the procedures to be followed by the last. Because the service must be operable on a multilingual basis, and is essentially designed for computer handling, a complete list of codes is given, any given attribute having the same code number in any language. These codes cover sponsorship, activities, functions, geographical coverage, working language, availability, output and subjects. Two further sections provide computer logic and computer search guides. Initially designed for use with

the programming languages COBOL and PL1, in both of which programs are already available, the system can be adapted to any modern computer, UNEP believes, in less than a week, and plans for conversions to other languages are already in hand.

In practice, a question is coded and passed to the computer, which selects those sources, details of whose stored or accessible information most closely fit the coded question. The answer describes the source (for example, government-sponsored laboratory, research organisation, university department), gives details of its coverage and lists those subjects most (and less) relevant to the query. The client then selects the source or sources that seem most likely to have the information he requires, and contacts them. The UNEP reckon that the cost, as far as the IRS is concerned, is about \$2 an answer. This has, of course, no connection with any fee payable by the user to the selected source.

Some 10 focal points are already operating the IRS, and another 40 or so should begin functioning within the next few months, including a regional one for the CMEA countries and, apparently, the USSR (although Hungary and Yugoslavia will have their own). The manual is available in English, French, Russian and Spanish. Alone of the official UN languages, Chinese cannot be used as it is not suitable for use with a computer. There are already translations into Danish, Dutch, German, Greek, and Italian. The UNEP has held the first of a series of training programmes in the use of the service in Nairobi. One will follow shortly in Geneva, on the use of relevant computer techniques, then two in Dakar (in French) and Mexico City (in Spanish).

The basic idea behind the IRS is to cover the environment in the broadest sense, recognising that people know their own disciplines but need information on others. Countries holding environmental information are not, they point out, necessarily the most advanced technologically; Kenya, for example, has much more experience of running National Parks than have many European countries. The aim at present is to run the service on the basis of the present manual for two years, then to appraise the situation to see what revision is needed. But as it stands, the IRS provides a good example of how a UN agency can function in the way most people think it should: as an international liaison office and clearing house, passing information between member countries or institutions within those countries, and so organised that everyone using the system knows what to expect and can be reasonably sure of getting it. □

As the much delayed jubilee celebrations of the Soviet Academy of Science finally approach, it is perhaps inevitable that the role of the scientist in Soviet society should become a matter for editorial comment in the Soviet press. The relationship between pure research and the immediate needs of the state plans is always a delicate one, and a saving clause referring to the ultimate benefit to the national economy is regularly included in the description of fund-hungry projects of the space programme. The closer connection between the pure scientist and the needs of industry has been one of the fundamental themes of the present five-year plan; this aspect of Soviet science is being stressed in the press preparations for the jubilee. The concept is one fundamental to Soviet thinking—Lenin himself envisaged a total reconstruction of the Soviet economy in which the Academy would play the major role in providing the Soviet Union with “the possibility of equipping itself independently with all the principal forms of raw materials and industry”. Seen in this light, the comment of a *Pravda* editorial (September 20) seems merely an exhortation to renewed efforts: “The duty of a scientist as a citizen is to appear as a champion of scientific and technical progress not only at the writing-desk or blackboard but also to fight for the cause outside the walls of his study or laboratory as well”. The reference recalls, however, the press campaign of September 1973 against Academician Andrei Sakharov, who was accused of betraying his chief duty as a Soviet scientist—that of being a patriot. Sakharov’s “offence” was, of course, his liberal political views, not an excessive preoccupation with pure science. Nevertheless the *Pravda* editorial does suggest that, once again, the slogan of patriotic duty is being used to urge scientists into a greater conformity with Party policy—in this case, the devoting of their talents to the immediate needs of the economy, even at the expense of potentially more valuable basic research—a somewhat ironic way, surely, of celebrating the 250th anniversary of the Academy of Sciences.

● Following recent speculation on the latest Soviet explorations in the Weddell Sea a number of statements made in the Soviet media and through the *Novosti* agency have set out the programme for the latest (twenty-first) Soviet Antarctic expedition, which is due to set out in October. This will include atmospheric sounding, the investigation of deep-level ice cores already taken by advance parties, oceanological investigation in the

Drake Straits, participation in the international South Pole Experiment (Polex South) and a massive survey of the mountainous hinterland of the Weddell Sea. The expedition, led by candidate of Geographical Sciences G. I. Bardin, is to be a large scale one, involving 255 scientists, a flotilla of five ships, including the flagship, the new research vessel Mikhail Somov, an Ilyushin-14 aircraft, four helicopters and five heavy tractors. It will also convey to the Antarctic a considerable amount of new equipment for existing Soviet bases.

Soviet diary

from Vera Rich



● During the past few years, a number of Franco-Soviet joint research projects have taken place, notably in the fields of geophysics and space research. Less publicised has been a biochemical experiment—perhaps because the research was carried out by an all-Soviet team from the Institute of Biochemistry of the Soviet Academy of Sciences, with France providing only some of the specimens. The purpose of the investigation was to establish the chemical constituents of the “bouquet” of champagne. The method used was that of gas-liquid chromatography; the test specimens were of both French and Georgian vintages. It was found that the bouquet is attributable to certain high-boiling-point constituents of fusel oils, notably terpenoid compounds of *cis*- and *trans*-farnesol, and also certain complex esters—ethyl linoleate, ethyl lactate, ethyl caproate, phenylethyl acetate and so on. This contradicts the previous assumption that the bouquet is attributable to highly volatile constituents. Small quantities of highly volatile compounds are, in fact, involved, but these form only a general ‘background’.

● The development of a silk industry independent of China was one of the great aims of the Byzantine emperors, who, when they at last obtained the

secret of silk-worm rearing, promptly made the industry an Imperial monopoly. From emperors to State Planning Committees seem a far cry, yet the Soviet Committee for Science and Technology has recently declared the establishment of native a silk industry to be one of its major inter-industry problems.

The new Soviet silk production is not to be based on the mulberry-feeding Chinese silkworm, but on the caterpillar of the oak egg moth, which is traditionally cultivated in Korea and northern provinces of India. After some 20 years’ research and selection, the Ukrainian Agricultural Academy has succeeded in producing a strain of insect which can be cultivated successfully in the harsher conditions of Poles’e and other areas of Ukraine. By means of hybridisation and selection, an insect has been produced with a life cycle compressed into a single season. Professor N. N. Sinitskii, who was responsible for the project, claims that now silk production need no longer be confined to the Central Asian Republics of the USSR, but can flourish “from the Carpathians to the Urals—wherever there are oakgroves”.

The effect on the ecology of Ukraine and the other western republics of the USSR as not been mentioned. However, in the Irkutsk provinces of western Siberia, another major research project has just been completed; a long term study of the best biological method for combatting a major pest of the Siberian forests—the Siberian silkworm.

● Thirty years ago, largely as a result of the havoc wrought by the Second World War, the European bison was a vanishing species. Now, as a result of careful conservation work in the Byelovezhchaya Forest (which is managed jointly by Poland and the Byelorussian SSR), it has made a remarkable recovery. Partly as a result of this capacity for survival, a team of zoologists in Vologda are attempting to produce a cross between the bison and domestic cattle. Several examples of the new hybrid have already been produced, and they have bison-like features, including increased body-weight, bluish-black hair, a slight hump, and straight sharp horns. The hybrid calves show considerable hardiness, being able to drink ice-cold water immediately after birth and having a considerably more rapid rate of growth during the first 10 days. The hybrid has proved to be fertile, and the first calving from the hybrid cows is due this autumn—after which the fat content of the milk can be established and compared with ordinary cow’s milk. It is hoped that the hybrid will preserve the high yield of the domestic cow.

correspondence

First-year ecology

SIR,—The teaching of ecology at university first-year level probably poses more problems than any other area of biology. Although in general there is agreement as to its desirability, opinions differ considerably as to the methods of implementation. Wide-spread public interest in topics such as conservation and pollution, reflected in pressure from students for courses in ecology, provide powerful reasons for attempting to overcome curriculum problems. We also believe that in an introductory biology course ecology should be presented not only as a subject in its own right but also as having important links with other fields of biology such as physiology, behaviour and genetics. Furthermore, it must be interdisciplinary, involving zoological and botanical aspects, as well as incorporating discussions of the role of micro-organisms in different ecosystems.

To meet these requirements at Bath, we have been reviewing the teaching of ecology at the first-year level in which there is provision for ten one-hour lectures and five two-hour periods of practical work. Our lecture/seminar programme centres around three principal themes—spatial distribution of organisms, the flow of energy in ecosystems and the gene as an element of continuity in populations. On the practical side we have accepted the fact that, at the most, only one field trip will be feasible and we exclude the possibility of vacation courses at this level. The main problem is the practical work which must necessarily be restricted almost entirely to the laboratory and be carried out in the minimum of time. It is also important that, being the first year, various investigations should work. We have therefore directed planning towards the possibility of using simulation experiments with living organisms, many of which have already been tried out in smaller and more advanced classes. The value of these experiments lies in the fact that they aim to reproduce and illustrate in miniature within the laboratory certain selected aspects of the real environment. Study involves not only qualitative description but also quantitative sampling and statistical treatment of results, together with simulation experiments covering such topics as succession, population growth, selection and the estimation of animal

numbers by means of mark, release and recapture experiments.

Simulation, however, has serious drawbacks since it demands extrapolation by the student from imitation to reality. Such an intellectual jump can pose problems. Although in the teaching of ecology, there is no substitute for the experience of a visit to a natural habitat, the introduction into the lecture component of visual aids, such as ciné films, still photographs and slides can help to bridge the gaps.

The inception of a new curriculum should also be associated with a fresh range of objectives some of which will bear on the cognitive field and some on the affective. It is not therefore enough merely to change the nature and quantity of the course content; the methods of teaching and learning must also be adjusted to suit the new requirements. This is particularly true in a complex subject such as ecology which tends to face students initially with a bewildering diversity of problems. Such needs have served to underline the changing role of the lecturer and tutor who acts not only in his traditional capacity of expositor, but also as guide, consultant and stimulator of discussion.

W. H. DOWDESWELL

I. C. POTTER

University of Bath

Cheating children

SIR,—I would like to comment on two letters in your issue of September 4 which are related to my work on metal bending. The first is that by Dr Pamplin and Mr Collins, whose interesting study of deception in spoon-bending leads me to ask what is the proportion of children who cheat in other forms of activity in which they are given the opportunity to deceive. I have also seen cases of obvious fraud in my initial tests and have not continued working with the subjects in such cases. I have found so far a ratio of no more than one in six of those who claim metal-bending powers (at least forty subjects) to have been able to achieve the effect in a manner which I cannot explain by deception. This is consistent with the results of Pamplin and Collins.

I would like to suggest that the criteria of bending might have been made more stringent before children were tested in this way, and even while the one-way mirror tests were being performed. Apparently some of the

children still claimed they could spoon-bend without cheating after they had been caught. They might still be able to do so, though only if the conditions are stringent enough will it be possible to get them to turn their attention to using any abnormal powers they possess. The measuring of pressure or at least having a strip of metal taped flat to a desk so it cannot be mechanically bent is necessary here.

In response to Mr Randi, who was very proud of his ability to be able to cheat all and sundry, may I say that his last sentence has remarkable hubris. I would be grateful to learn of Mr Randi's scientific qualifications before I can accept it, and I echo the apt words of the great scientist Sir William Crookes, a past President of the Royal Society, by insisting that when it is necessary to trust my senses I maintain that a physical enquirer is more than a match for a professional conjurer.

May I also recommend to Mr Randi the report of his fellow magicians from Atlanta, A. Zorka and A. Dickson who tested Mr Geller under conditions they felt excluded fraud.

Yours faithfully,

JOHN TAYLOR

King's College, London, UK

Assays

SIR,—With reference to the article of A. Dorozynski (September 11), I would like to make the following comments:

1. The mutagenicity assay, elaborated by Dr Bruce Ames, has been used by the IARC in Lyon for three years within the framework of a continuous collaboration with Dr Ames. Dr Bartsch of the Unit of Chemical Carcinogenesis of the IARC has devised some modifications of the original procedure which have allowed an improvement of the testing efficiency.

2. The IARC is collaborating, and is certainly willing to continue and expand its collaboration, with a number of national laboratories, including the Institut Pasteur in Paris, on the development of screening tests to detect chemical mutagens and/or carcinogens.

3. The IARC is not, and will not be, involved in the commercial dissemination of the "mutatest".

Yours faithfully,

L. TOMATIS

*International Agency for Research on Cancer,
Lyon, France*

news and views

In the world of professional archaeology of the 1920s and early 30s, the fact that you were a 'Glozelian' or 'Anti-Glozelian' determined who were your friends; this was particularly significant in an academic discipline not unknown for acrimonious controversies. The reason for this in-fighting was based on a reported discovery of artefacts which to most archaeologists were—and to many still are—unacceptable and were therefore labelled as fakes. The first pieces were reported in 1924 by a local farmer, Emile Fradin, who is still very much alive living in the same farmhouse at Glozel, an isolated village south of Vichy in France; these discoveries were quickly followed by many more undertaken by a M. Morlet, a doctor from Vichy. What caused the furore was the fact that these objects were like nothing seen before anywhere in the world and covered an amazing spectrum of different types. There were clay tablets with mysterious incised inscriptions in an unknown language, jars and other ceramics with and without inscriptions, pottery phallic symbols, animals inscribed on bone and pebbles. None of these objects was recognisable archaeologically and even if accepted as something new, many found it impossible to reconcile the different periods represented apparently cheek by jowl; for instance what might conceivably be Neolithic incised stones were apparently found in the same context as clay tablets with inscriptions, which, some said, represented early Iberian scripts.

The controversy raged more or less continuously until the War in 1939. During this time two international commissions of inquiry sat and pronounced exactly opposite decisions and some five law suits were fought with varying results. In Britain the balance against the Glozelians was heavy although on the continent such eminent archaeologists as Professor Reinach were strongly opposed to the notion that deliberate forgery had taken place.

There the matter rested until 1974 when Glyn Daniel, Professor of Archaeology at Cambridge University, decided to dot the i's and cross the t's in his lecture on archaeological fakes and forgeries; he would have a few pottery samples from Glozel tested using the thermoluminescent (TL) technique in order to illustrate their modern date. Exactly the opposite result transpired—the TL measurements clearly indicated

The Glozel affair

from E. T. Hall

that the ceramics were not modern, but seemed to have been manufactured in the Gallo-Roman period (*Antiquity*, December, 1974).

These results, which were later more thoroughly confirmed by further measurements, have again divided the archaeologists. To some the antiquity of the ceramics in particular is no great surprise, although the admixture of these ceramics in the same context as other less acceptable material, such as engraved bone and pebbles of apparently Neolithic date (which are less amenable to dating techniques), makes the unravelling of the whole story very difficult. To other archaeologists the affair is still a distasteful hoax and TL dating must be in error; for them it is similar to telling a physicist that the laws of thermodynamics are no longer valid and, not surprisingly, they find it difficult even to listen.

Where then can the explanation lie? At the symposium on Archaeometry at Oxford University in March, details of the work undertaken by McKerrell at Edinburgh and Mejdahl in Denmark were given, and discussed at length by both physicists and archaeologists. There can now be little doubt that the ceramic material is not modern; preliminary dating results on the bone using the ^{14}C technique tend to support the TL measurements. It has been suggested by some that the ceramics, which were low-fired, could be ancient material such as tiles which had been reshaped and inscribed recently. Though this is a possibility with some tablets, with others it is ruled out because on close examination small bubbles of vitrification can be seen in the grooves of some of the inscriptions. Others have suggested recent deliberate irradiation by X rays or γ rays to give the correct TL dates; such possibilities are ruled out by comparatively simple tests which have indeed been carried out. However it is generally agreed that the archaeology associated with the digging is most unsatisfactory. No properly controlled digging took place except for attempts by both commissions of inquiry of a very limited kind and when 'planting' of finds seemed a distinct

possibility. There is, therefore, no scientific evidence that all of the objects were in fact from the same stratigraphical context or even from Glozel itself.

It is important in these discussions to differentiate between authentication and dating by TL measurements. When a ceramic object has been removed from its immediate surroundings and has been kept in a museum for some years the extent of the radioactive bombardment from the surrounding soil (as opposed to the internal contribution) becomes a matter of conjecture. Dating by TL measurements of such an object must then have much wider limits than when the precise environment is known and an accuracy of $\pm 10\%$ or better can be expected; when the burial environment is unknown the dating accuracy must be subject to greater errors, although in the instance under discussion the approximate level of soil radioactivity would be known provided, of course, that the objects were in fact excavated from the Glozel area. In the case of authentication however we are dealing with a different order of magnitude; in this case we are deciding between an age of 50 and at least 500 years: an error of 1,000% or more. The Glozel measurements would seem to show without doubt that the ceramics are not modern fakes, but it might be a little rash to differentiate with any complete confidence between, say, a Gallo-Roman and a mediaeval provenance. The idea of a mediaeval date becomes a clear possibility after the work of Huxtable and Aitken reported in the latest issue of *Antiquity* (September 1975), albeit on a single specimen. Such a date is perhaps more likely since an archaeologically acceptable mediaeval kiln was discovered on the site. They also point out that McKerrell and Mejdahl's results are based on only five dates and it will be interesting to see, when full publication is made, whether all the objects sampled fall into a coherently dated group, taking into account the wide range inherent in this type of TL measurement.

How does TL dating come out of the controversy? There have during the past four years been some dramatic exposés of modern fakes which had been unsuspected until the advent of TL dating; many Tang wares from Hong Kong, Hacilar from Turkey, Amlash from Tehran and massive Etruscan terracottas from Italy have

all been shown to have been manufactured during the past few years on a large scale and are (or were) decorating shelves in private collections or public museums around the world. These results are now accepted almost unanimously by the relevant authorities and there have been no obvious instances where results undertaken by a reputable laboratory have been shown to be in error as far as authentication is concerned; in other words, so far TL authentication has been shown over many hundreds of samples to be reliable. It would seem unlikely that the Glozel ceramics should be an extraordinary exception to this story of reliability particularly since they do not exhibit any anomalous behaviour during measurement and give entirely normal TL glow curves.

A number of the archaeological objections to the authenticity of the site would be removed if it were found that certain of the objects

were genuine and some false. For instance; one might postulate that the higher fired tablets were genuine, but the weird face urns, phallic symbols, bone and pebble carvings were not; if the ceramics in the latter category had been fabricated from tiles or bricks fired in antiquity and reconstituted, this could give an explanation of their apparent ancient TL date. Measurements being undertaken at Oxford using archaeomagnetic techniques may give some clues as to whether this is a possibility.

It is to be hoped that the projected scientific re-excavation of the site by French archaeologists will proceed apace—a preliminary magnetometer survey has already been undertaken. Although the site has been extensively and randomly dug by many different excavators, if the site is genuine, some objects must remain and their archaeological contexts may help to explain this perplexing problem. □

Polymorphs and amorphs

by Robert W. Cahn

THE central puzzle about glasses is this: why do some melts form glasses on slow cooling while others do not? As with all such broad questions, the golden nugget of understanding recedes further and further as hypotheses pile up. If an explanation is attempted in terms of the viscosity/temperature relation, then one wants to know what determines this reaction; if in terms of critical nucleus size and diffusion rates, then again one asks how these are related to chemical composition. As more and more unconventional glass-formers are discovered, it becomes increasingly difficult to establish what chemical or structural features they have in common. A radically novel hypothesis therefore deserves close attention.

Such an hypothesis is advanced in this issue of *Nature* (page 370) by C. H. L. Goodman. The central observation on which his hypothesis is based is: "A surprisingly large number of the systems which form glasses have as a major (sometimes the only) constituent, a material that exists in two or more polymorphic crystalline forms which differ but little in free energy", and he goes on to exemplify silica, BeF_2 , PbO , As_2O_3 , TiO_2 , Se , S and CdP_2 . He then asks whether polymorphism could be a necessary condition for a material to be a glass-former. The rest of his extremely interesting and well-documented paper is devoted to exploring, admittedly in purely qualitative terms, the implications of his novel hypothesis.

He suggests that, above the glass transition temperature T_g , transient

"flicker-clusters" of all possible polymorphs should coexist, as happens for instance in liquid water. The crucial point here is that none of these clusters can reach a viable size for crystal growth, because the various clusters of polymorph A would be impeded by the clusters of polymorph B, and in particular the separate A clusters could never achieve mutually epitaxial orientation relationships. As the melt cools, eventually the clusters become firmly bonded across parts of their surfaces, leaving liquid-like material in other, unbonded interstices. This stage corresponds to T_g . The role of the minor additives that assist glass formation might well be to concentrate in the residual interstices and reduce the strain resulting from differential thermal contraction of the distinct A and B clusters. (Goodman regards it as an important test of any hypothesis whether it can interpret the important role of some glass-modifier additives which have a major glass-stabilising effect in quite small concentrations.)

Goodman suggests various experimental tests of his ideas: for instance, ways are available to check on the coexistence of microcrystalline clusters and liquid-like interstitial layers. Also, recent work on the radial distribution function in vitreous silica, combined with dilatometric and high-pressure studies, have yielded evidence for the simultaneous presence of several micro-polymorphs. He goes on to point out certain categories of materials which on the basis of his hypothesis should not form glasses: there are thus the begin-

nings of a predictive capacity for the model.

Particularly interesting is the final section of Goodman's paper, where he suggests possible practical implications of his ideas. Thus, the solid cluster/liquid layer model implies that near T_g a glass might behave as an effective absorber of gases, like a zeolite, and points out that this is consistent with the known action of glass films on the surface of semiconducting devices. He suggests that carbon melts containing 'solvent catalysts' such as iron or nickel (used for diamond synthesis) might be capable of being quenched into the glassy state. Finally, he suggests that Griffith cracks, which determine the tensile strengths of glasses, might be controlled by the microstress systems and chemical heterogeneity of his postulated heterocluster arrays. He does not say it, but it might well be that the scale of the cracks (and hence strength level) would be determined by the size distribution of the clusters, and one then becomes interested in what determines this distribution!

One feature of many oxide glasses to which Goodman does not address himself is the observation that many (perhaps most) such glasses undergo liquid-liquid separation above T_g : electron microscopy and X-ray small-angle scattering both show that disperse drops of liquid separate out in a matrix of liquid of distinct chemical composition. It is not immediately clear whether Goodman's hypothesis would imply that each liquid phase needs to break down into arrays of two (or more) types of polymorphic microclusters, or whether it suffices if one phase does so. In any case, it would appear that in view of the prevalence of liquid separation, Goodman's ideas imply that there are often two distinct levels of structural heterogeneity in a stable glass.

This paper will suggest to the reader all sorts of new consequential hypotheses and various kinds of experiments to follow them up. Whether or not time fully confirms his ideas, his paper will undoubtedly prove to be of seminal importance. □

Bølling interstadial in Britain?

from Peter D. Moore

THE elucidation of climatic fluctuations at the close of the last (Devensian) glaciation in Britain is fraught with difficulties (see *Nature*, 251, 185; 1974). One of the major controversies centres upon the events leading up to the so-called Allerød interstadial; in continental Europe a warm period (termed the Bølling interstadial) is believed to have preceded the Allerød,

and there is some indication of another warm period before the Bølling, called the Susaca interstadial (van der Hammen and Vogel, *Geol. Mij.*, **45**, 33; 1966). In Britain, evidence even for the Bølling is scant and it is widely believed that its influence upon contemporary vegetation was of such a nature that it cannot be distinguished from the succeeding Allerød. Not all palaeoecologists are of that opinion however.

Last year Burrows published the results of his analysis of plant macrofossils from a late Devensian lake site at Nant Ffrancon in North Wales (*New Phytol.*, **73**, 1003; 1974). This site had previously been worked upon by Seddon (*Phil. Trans. R. Soc.*, **B244**, 459; 1962), who described a typical sequence of sediments and fossils suggestive of a cold/warm/cold development in late Devensian climate. The new core studied by Burrows, however, revealed a rather different sequence. The organic sediments, often indicative of the Allerød interstadial in British sites, was found, and above and below it were clays with a stony content and with macrofossils indicative of colder conditions. But within the lower clays, about 26 cm below the supposed Allerød, were silty muds, rich in organic matter, and containing plant fragments indicative of relatively warm climatic conditions, including tree birch and *Filipendula ulmaria*. Naturally, this stratigraphic profile, with two organic layers, leads one to speculate that the Bølling interstadial has at last been found in Britain.

It seems very strange, however, that the Bølling should make its effects felt in the mountainous regions of Snowdonia, and yet should not be evident in the many lowland sites which have been studied. In support of his contention, Burrows quotes the data of Crabtree at Cors Geuallt, just 10 km from Nant Ffrancon. There, a similar but very thin, organic band was reported in the pre-Allerød clays (*VIII^e Congrès INQUA, Paris*, **1**, 217; 1971), but the arrangement of pollen sampling within the core in question does not permit its clear interpretation as the product of a Bølling interstadial.

The interpretation of cores, either in temporal or climatic terms, on the basis of their stratigraphic sequence alone is a risky business. One needs absolute dates, in this case radiocarbon dates, before such a sequence can be understood. Burrows has recently published some dates from his Nant Ffrancon core (*New Phytol.*, **75**, 167; 1975), but unfortunately the difficulties are still not resolved. Of the four dates he has obtained, he is forced to dismiss the lowermost two as gross underestimates, possibly resulting from contamination. The lowest acceptable

date comes from the basal layers of the upper (supposed Allerød) organic layer, and is $11,900 \pm 500$ b.p. The commencement of the Allerød is conventionally dated at 11,950 b.p. (Godwin and Willis, *Proc. R. Soc.*, **B150**, 199; 1959), so Burrows' date confirms these deposits as belonging to the Allerød interstadial, as it is normally understood in the British context. Although this date still allows the Bølling interpretation which Burrows sets upon the lower organic layers, it does nothing to confirm this. When one takes into account the possibility of rapid sedimentation of the intervening clays and the large standard error of the date quoted, it is still possible that the lower muds also belong to the traditional Allerød.

The case for an undisputed British Bølling therefore still remains open. A more important fact emerges from this involved controversy, however. Despite a plethora of regional information on late Devensian sequences within the British Isles, we still have no type section at which our distinctively British sequence can be defined. No amount of pro-European feeling can excuse the casual and largely inaccurate use which we make of such terms as 'Allerød' and 'Bølling', which have only been defined adequately in their continental context. An intensively studied British type site might at least remove some of the suspended sediment from these currently disturbed and cloudy waters. □

Photoperiodic regulation of insect growth

from our Insect Physiology Correspondent

THE arrest of the process of growth in insects that is known as diapause may occur at different phases of embryonic development, during larval growth, in the pupal stage or in adult reproduction. Diapause used to be defined as an 'inborn' arrest because it appeared to be uninfluenced by environmental changes. But that was because the 'sensitive period', during which induction of the arrest takes place, often occurs long before the actual arrest appears, and the inducing factor was therefore overlooked. In recent years increasing attention has been given to the nature and regulation of the sensitive period itself. A recent paper by D. S. Saunders (*J. Ent. (A)* **50**, 107; 1975) deals with these phenomena in the flesh-fly *Sarcophaga argyrostoma*; it is an instructive short paper because it reviews and confirms earlier observations on this insect (and other insects), contributes some important new discoveries, and generally illustrates the modern approach to the subject of diapause.

The flesh-fly *Sarcophaga* has a diapause controlled, as usual, by photoperiod. Sensitivity to photoperiod begins while the embryos are within the maternal uterus (in these flies the eggs hatch within the common oviduct) and continues throughout larval development, to end at the time of puparium formation. A short daylength of less than 14.5 h in 24 h gives a high proportion of diapausing pupae: larvae raised at a long daylength exceeding 14.5 h give rise to pupae with uninterrupted development. As in other insects a definite number of short- or long-day cycles is required to induce the necessary switch. As the author had earlier shown, these two variables (1) the length of the sensitive period, that is, the length of larval life and (2) the number of short-day cycles required to raise the proportion of diapause pupae in one day's batch to 50%, have different temperature coefficients.

The length of larval life is highly dependent on temperature ($Q_{10}=2.7$), whereas the 'required day number', as defined above, is largely compensated for temperature change ($Q_{10}=1.4$ or less). Consequently, at high temperature (26 °C) the larvae reach puparium formation and their sensitive period comes to an end before the required number of short-day cycles has been experienced and development proceeds without interruption. Whereas, at a low temperature (16–18 °C) the sen-



A hundred years ago

THE *Photographic News*, in speaking of "Photography and the Illustrated Press," gives some examples of the extent to which the latter is now dependent on the photographic art. The *New York Daily Graphic*, besides often executing its pictures from photographs, employs a photo-mechanical process in the production of some of its work. At the office of the *Moniteur Universel*, which is one of the most extensive printing and publishing establishments in France, arrangements are being made for large photo-printing works, as well as for producing coloured pictures by M. Leon Vidal's photo-chromic process. In this country photography is used to aid the artist in sketching to a great extent. One of these days, no doubt, the *News* believes, we shall have our papers illustrated by photographs *pur et simple*, but even now photography has far more to do with the execution of the illustrations in our journals than most people may be aware of.

from *Nature*, **12**, 503, October 7, 1875.

sitive period of development is prolonged, sufficient short-day cycles can occur and the incidence of pupal diapause is high. Moreover, the length of larval life is shorter at long daylengths than at short daylengths and this will reinforce the effects of temperature.

The author now tests this interpretation by exposing larvae to short-day cycles (LD 12:12) and borderline temperatures of 18 and 20 °C, and then varying by other means the duration of larval development and so the sensitive period. The most effective means of accelerating puparium formation, and so curtailing the sensitive period, was by overcrowding larvae in a limited quantity of meat; and as predicted this reduced the incidence of pupal diapause. (Premature extraction of third instar larvae from the culture, or exposure to pure CO₂ for 24 h had comparable effects.) The sensitive period was lengthened by allowing larvae to wander in wet sawdust, which defers puparium formation; this treatment increases the incidence of pupal diapause.

The theory that insect diapause results from an arrest in secretion of the hormones necessary for growth (or reproduction) was put forward more than 40 years ago and is now fairly generally accepted. Precisely how photoperiod controls hormonal activity is not known. In general it is thought that long or uninterrupted nights are inductive in the diapause-promoting sense, whereas short or interrupted nights are inductive in the development-promoting sense. There is evidence that both effects are accumulated by the photoperiodic counter, the former to a threshold at which, for example, brain hormone secretion is suppressed, the latter to a point at which secretion is released, thereby leading to ecdysone secretion by the ring gland, puparium formation, growth, and eclosion. What is it that is being summed? The author argues convincingly that the summation of successive photoperiods cannot be the summation of cryptic effects of ecdysone. It seems more likely that the mechanism for summing photoperiodic cycles lies in the brain itself where the release of the brain hormone is regulated. □

How birds reach home

The 14th International Ethological Conference was held at Parma, on August 27–September 5.

from John R. Krebs

THE problem of how migrating birds and homing pigeons navigate still

remains unsolved in spite of intensive research efforts in the past few years. Over twenty years ago, Gustav Kramer suggested that in order to fly from an unfamiliar place to a geographically distant home site, a bird would need information analogous to a map (on which to read its own position and that of home), and a compass (to choose the appropriate direction indicated by the map). It is now well established that both migrating song birds and homing pigeons (on which much of the experimental work is done) have up to three types of compass—based on the Sun's azimuth, star patterns and the resultant of the Earth's magnetic field, but the nature of the map remains elusive.

F. Papi and his colleagues from Pisa presented a synthesis of their experimental evidence supporting the idea that pigeons use an olfactory map of windborne smells to home from an unfamiliar release site. The results are impressive. In one experiment three groups of pigeons were reared in outdoor aviaries side by side. Two of the groups were exposed to abnormal wind directions by an arrangement of screens that deflected the wind through 90° clockwise or counterclockwise, and the third group acted as a control. When the pigeons were released 105 km from home, the control group headed straight home, the group exposed to clockwise wind rotation headed off 65° counterclockwise from home, while the counterclockwise group deviated clockwise by a similar amount. In a second series of experiments, pigeons were raised in outdoor aviaries and exposed to artificial winds from the North or South bearing the scent of olive oil or α -pinene. The birds were later released 26 km West of home, and flew South if a drop of 'North-wind' scent was placed on their nostrils before release, while they flew North if treated with the 'South-wind' scent. These and other experiments all suggest that the birds learn to associate particular scents with winds coming from particular directions towards the home loft, and that they use this information together with the concentration of scent at the release site, to construct an olfactory map showing the position of the release site in relation to home.

Although the Pisa group's results look convincing, W. T. Keeton (Cornell University) reported that he was unable to replicate them, and the reason for this discrepancy is unclear, although as one Italian delegate suggested, the Italian countryside, redolent with the aroma of olives and garlic, may be more conducive to olfactory navigation than are the extensive deciduous forests of upstate New York. In any case, it seems much less likely that an olfactory map would be used by long distance migrants travelling thousands of miles from

northern temperate regions to the tropics. Many of these birds travel at night, and for some years it has been known that they use the pattern of the stars as a compass. W. Wiltschko (Frankfurt University) reported some very neat experiments showing that the star compass is 'set' according to the magnetic compass that these migrants also possess. Wiltschko recorded the spontaneous migratory fluttering movements of garden warblers and robins in small circular cages. When the birds were allowed to see the sky on a clear night in a normal magnetic field, they fluttered in the usual migratory direction, roughly South in the autumn and North in the spring. When magnetic North was artificially rotated by 120°, the birds correspondingly altered their heading, even though the stars were still visible. Robins took longer to adjust than garden warblers, so they seem to take more notice of the stars. Wiltschko then showed that the robins could be trained to use a totally artificial pattern of 'stars' as a compass, if they were first shown the pattern in association with the Earth's magnetic field. The magnetic field is used to 'calibrate' the star pattern, which can then be used on its own. One may well wonder why the birds use a star compass at all if it has to be set with reference to a magnetic compass; perhaps it requires longer to 'read' the magnetic compass, so that the visual compass is used for quick reference while the bird is in flight. Finally, the big remaining mystery surrounding the magnetic compass, is how the bird's sensory system detects the Earth's magnetic field. □

Conservation of marmosets

from J. P. Hearn

An international conference on the Biology and Conservation of Marmosets convened by the National Zoological Park, Smithsonian Institution was held at Front Royal, Virginia on August 18–21.

MARMOSET monkeys are becoming more common in laboratories and rarer in the wild. The potential of marmosets as models in human oriented studies in immunology, virology, reproduction, contraception, teratology and other fields, is just starting to be recognised, but already some species are impossible to obtain and several South American countries have banned the export of marmosets completely.

Recently, sixty scientists from the United States, South America and Europe participated in an International Conference on the Biology and Conservation of Marmosets and the picture that emerged gave cause for concern.

Several species of marmosets—the lion tamarins, the buff headed marmoset, the white eared marmoset and the cotton topped tamarin (*Leontopithecus* species, *Callithrix flaviceps* *Callithrix aurita* and *Saguinus oedipus oedipus*)—are threatened so severely that they are near extinction. The major threat to these animals is the clearance of their natural forest homelands. The lion tamarins from Brazil are now dependent on only 2% of their original natural range and not more than 600 are thought to survive in the wild.

Three suggestions were made to rectify the situation: A total ban on the export, shooting, or interference with the endangered species; urgent studies in the wild by census and observation; and careful management of the remaining home environment. These animals depend on particular types of trees for food and nesting holes. Planting of such trees, with shelter holes artificially bored in them if necessary, and a supply of additional foods, would assist the survival of these animals in the present unnaturally reduced environments to which they are restricted. As any delay in the application of effective measures may well prove disastrous, the conference asked for assistance from conservation organisations and the governments of the source countries.

Marmosets used in research are usually the common marmoset, the saddle-backed tamarin and the moustache tamarin (*Callithrix jacchus*, *Saguinus fuscicollis* and *Saguinus mystax*). These are not immediately threatened but may become so as growing research needs outstrip the replacement of natural wild stocks. The threats to their continued supply from the wild are the appalling losses suffered at present during shipment from the wild to the user countries and the accelerating rate of deforestation in developing South American countries.

In this case there are several longer term solutions that can be applied. Marmosets, and in particular the common marmoset, are prolific breeders in and out of captivity. They can be maintained in self-replacing colonies that are inexpensive to run compared with colonies of the more conventional, larger laboratory primates. Therefore, breeding centres should be established on a large scale commercial basis both in the countries of origin and the countries where marmosets are used in research. Laboratories should also develop colonies that are self-replacing in order to become independent of supplies from the wild.

In the shorter term it is essential that the conditions in which marmosets are held between capture in the wild and arrival in the laboratory be greatly improved. Of necessity this control must be applied in the source countries; by the rigorous application of export quotas and by inspection at the export centres. At present only about 10% of marmosets caught in the wild survive the various stages necessary before they become established in the laboratory. This has resulted in an unreal escalation in demand. The situation could easily be improved as marmosets are amenable and adapt very easily to captivity if given the right conditions.

At a time when the need for primates in research is growing and supplies from the wild are dwindling, many scientists are looking to the smaller new world monkeys as an alternative to the more conventional macaques. The marmosets are receiving particular attention as they are small, inexpensive, easy to manage and breed well in captivity, but if their exploitation is not more carefully managed than it has been in the last five years there will be disastrous consequences for the wild populations. □

New light on luminescence

from G. F. J. Garlick

An International Conference on Luminescence was held in Tokyo on September 1–5. The full proceedings will be published in an issue of the *Journal of Luminescence* in 1976.

New lamps for old might have been the cry at many previous luminescence conferences, particularly because of the developments in fluorescent lighting, electroluminescence and cathode ray tube phosphors. This year in Tokyo, however, it was a different transition. Having seen its offspring, the laser, safely launched, luminescence research is utilising the high intensity and short pulse lifetime features of lasers to enter a new phase. It is now possible to look at the very early stages of excitation and at the fascinating effects of high excitation densities. It is no longer legitimate to separate the emission processes from absorption and excitation and one can hardly sustain the definition of luminescence which distinguishes it from the Raman effect. The invasion of the pre-relaxed states of both inorganic and organic systems by experiment and theory was well described in the various review papers at the conference. The process of luminescence is now

analogous to the 'compound nucleus model': the luminescing system interacts coherently with the exciting radiation field and relaxes by various channels. K. K. Rebane (Institute of Physics, Academy of Sciences of Estonia, USSR) showed that it is possible to look at the unrelaxed states at ordinary excitation levels in the steady state but there seems to be no doubt that picosecond techniques and high intensities are needed to sift out the detailed stages from coherence interaction to the dephased, relaxed state. As exemplified by the large number of original contributions, high intensity studies reveal, through their characteristic luminescence spectra, new entities such as the interacting exciton-photon—the polariton; the excitonic molecule or bi-exciton and, given the right physical conditions, the condensed exciton system—the Bose droplet. The latter has been detected in sizes up to 1 mm as reported by C. D. Jeffries (University of California, Berkeley). Although such phases are not yet very evident in the organic systems, the spate of papers on exciton migration and interaction in aromatic crystals leaves no doubt that larger co-operative entities are likely to be found there also. The larger-than-usual collection of papers on organic crystals had a unifying influence on the meeting which should be strengthened in the future. There was also an important group of papers from P. M. Rentzepis (Bell Laboratories, New Jersey), R. Kopelman (University of Michigan) and V. J. Koester *et al.* (Purdue University) which showed that picosecond and other spectroscopic-time resolution techniques can be applied to luminescence and related optical processes in chlorophylls with a good chance of unravelling the initial excitation and transfer mechanisms in photosynthesis.

The radiationless transitions which detract from luminescence efficiency are usually only indirectly observed through quenching of luminescence. M. B. Robin (Bell Laboratories, New Jersey) has, however, detected the heat pulse from a non-radiative transition by means of the 'second sound' pulse propagated in superfluid helium and recorded by a superconducting lead bolometer. In a different experiment at helium temperatures, N. Riehl (Technical University, München), has shown that it is possible to detect single phonon propagations in crystals by their activation of electrons from very shallow traps.

Among new luminescent materials reported were some stoichiometric rare earth compounds (such as $\text{NdP}_3\text{O}_{11}$) developed by M. G. Danielmeyer (Max-Planck-Institut für Festkörperforschung, Stuttgart) needing no impurity doping and having high efficiencies as

laser crystals. M. Genet *et al.* (Institut de Physique Nucléaire, Orsay) described a ThBr₄ phosphor, efficient as a scintillator and capable of excitation by a β source to give enough light to a photovoltaic cell to provide an energy convertor of about 0.3% efficiency! To these we may add recent phosphors, reviewed by A. L. N. Stevels (Philips Research Laboratories, Eindhoven) which may be used to give three component fluorescent lamps, substitutions for the sulphide phosphors of cathode ray tubes and for the current X-ray intensifying screens. All of these materials utilise the features of rare earth activators in a variety of host crystals.

Among new effects reported at the meeting, E. Nakazawa (NHK Broadcasting Science Research Laboratories, Tokyo) described photon absorption and emission at twice the energy of the individual level separations of cooperating pairs of rare earth ions in an yttrium phosphate crystal. One might ask, what has happened to the former extensive interest in electroluminescence? There were some papers on the subject at the meeting but lack of financial largesse has forced research into a narrower range of materials, notably into studies of the deep lying band gap states in GaP, GaAs and their alloys and into other features which might be holding down the efficiency of light-emitting diodes. This basic work seems preferable to free ranging over every possible electroluminescent system although there is still an unsatisfied hope of a breakthrough in high band gap crystals like ZnS and ZnSe.

The institution of informal discussion groups on specific topics, such as picosecond techniques, proved very popular. Organisation in all respects was excellent and set a high standard for emulation at the next conference to be held in Paris in 1978. □

Neutron diffraction in Holland

from G. E. Bacon

A meeting on New Methods and Techniques in Neutron Diffraction was held on August 5-6 at the Reactor Centrum Nederland near Amsterdam. Papers presented at the meeting will be published as a report of the Reactor Centrum Nederland.

AFTER attending international meetings on neutron diffraction for a quarter of a century it was a new experience to go to one at which only 8, out of an

audience of 127, came from the United States. At first sight such a circumstance at the Reactor Centrum Nederland at Petten, near Amsterdam, may be thought simply to underline the increasing financial difficulties which face those who have to travel large distances to keep in touch with their scientific colleagues. More fundamentally the unusual ratio reflected the dividend which is now being paid for the Franco-German, and latterly and more tardily the British, collaboration in the High Flux Beam Reactor at the Institut Laue-Langevin in Grenoble. More specifically, much of the discussion at this meeting reflected the success of the German investment in guide tubes and other neutron optical devices inspired by H. Maier-Leibnitz. The papers were restricted to topics relating to elastic scattering only.

The six sessions made it clear that the number of available techniques in this field is immensely larger than it was 5 or 10 years ago, mainly because a high flux makes a practical possibility of many methods which developments in computing had made possible, in principle, some years earlier. The employment of position-sensitive detectors, contrast-variation in small-angle scattering by biological substances, polarisation analysis and powder-profile refinement are now producing scientific results concerning interesting materials, rather than demonstration experiments. For example, in protein molecules, by labelling different parts of the molecule with selective deuteration, it is possible to do much more than determine an average radius of gyration. By small-angle scattering also (G. Lippmann, Institut für Festkörperforschung der Kernforschungsanlage, Jülich, BDR) the dimensions of the nickel-depleted zones in invar alloys have been found. By profile-refinement, structures have been determined (J. C. Taylor, Chemical Physics Division, Lucas Heights, Australia) for a wide range of uranium compounds which are very intractable for growing and maintaining as single crystals. D. Hohlwein (Institut Laue-Langevin, Grenoble) indicated the practical possibilities of photographic detection, showing, for example, neutron Weissenberg photographs of the superstructure reflections from the mineral labradorite. M. Schlenker (Laboratoire du Magnétisme, CNR, Grenoble) showed how the penetrating ability of neutrons enabled topographic studies to be extended to the interior of crystals, thus effectively studying a section through a crystal without the disadvantage of having to destroy it by cutting a slice.

Rather belatedly perhaps, work has thrived on the use of pulsed sources, including particle-accelerating machines, for neutron diffraction experi-

ments using time-of-flight techniques. This is not solely because of the advantages which the fixed geometry yields for the production of cryostats, furnaces and high-pressure cells but probably with an eye to the future, where the next generation of neutron sources may well not be the conventional reactor, but some kind of neutron booster or spallation machine capable of increasing neutron beam intensities by another order of magnitude. □

From babble to Babel

by Miranda Robertson

The Third International Child Language Symposium was held in London on September 3-5.

It was linguistic theory, and in particular the transformational grammar of Noam Chomsky, that led to the recent surge of interest in child language; but while the interest is unabated, it seems that the influence of linguistics has begun to wane. Chomsky's view that the deep structures of his transformational grammar reflect the innate organisation of the neural substrates for language led researchers to look for evidence in the earliest utterances of infants and in fundamental similarities in the first sentences of children speaking widely different tongues. It is now generally felt however that transformational grammar has failed to illuminate either.

Richard Cromer (MRC Child Development Unit, London), as chairman of the session on the acquisition of syntax, attempted to categorise the advances and retreats. For example, as he pointed out, there is less work on syntax but more on semantics. That is partly because of a belated realisation that it is impossible to separate the two, since in order to look at how a child starts to use syntax to express his meaning it is necessary to know what meaning he intended to express. And it is the need to understand the child's semantic intentions that has led to another important reorientation (or in Cromer's terms, an advance)—from the dogmatic to the pragmatic approach. That has led to the use of the ethological research strategy promulgated perhaps most notably by Professor Jerome Bruner (Oxford University).

What, then, have psycholinguists learned by watching the emergence of infant speech in its natural habitat? Elizabeth Bates and her colleagues (University of Rome) have been able to follow the progression from gesture to first word in a longitudinal study on Italian children from the age of 2

months. It is commonly accepted that the single-word utterances of infants can be divided into imperative and declarative modes, at a stage at which differences in intonation and gesticulation have to substitute for syntax to indicate whether the intention is to demand something or merely to point it out. Bates has been able to trace the development from imperative and declarative gesture alone to the later association with words.

As any mother knows, and as Linda Ferrier (University of Bristol)—who is one—pointed out, the meanings of babies' first words are frequently complex and usually idiosyncratic, because the child has to encode in one word the semantic content of a sentence and he generally does so by presuming on his shared experience with his mother. For example, Ferrier's own child used the word "cat" to request a drawing, because cats were what Ferrier habitually drew.

Because the single words of small children are so loaded with significance, they have come to be known in the jargon of the field as holophrases, whose interpretation is central to an understanding of how children develop more complex linguistic skills. Dr Patricia Greenfield (University of California, Los Angeles) therefore aroused particular interest with a report of preliminary observations on two children (one hers) on how a child selects one of several appropriate single words to utter in a given situation. Her thesis is that the decisive factor is the word's information content, somewhat intuitively defined in terms of novelty or unexpectedness. The child will thus draw attention to the aspect of a situation which surprises him and may be new to you; which seems a sound functional basis for communication but has no obvious linguistic implications.

Linguistic factors in the acquisition of speech proved generally elusive. Elizabeth Sharpless (City University of New York) found linguistic complexity accounted less well for the order of acquisition of the personal pronouns than did the part played by the child in her experiment. The crucial factor was whether he was a participant in the dialogue, or an onlooker.

H. H. Chipman (Geneva University), following the development of comprehension of possessive pronouns, remarked that at the earlier stages the child's interpretation of a sentence is quite independent of pronoun reference and seems to be based entirely on his presuppositions about the world. That sort of work exposes the need to consider a child's linguistic development in the light of his cognitive development in general, and David Ingram (University of British Columbia) and Bates and

her colleagues have been attempting a systematic study of the relationship between linguistic development and Piagetian cognitive stages. That has involved both of them in some redefinition of one or two of the stages but does not threaten Piaget's view that language develops as part of the child's general capacity for symbolic expression.

Comparisons on the speech of children of different nations, too, yield cognitive rather than linguistic universals, according to D. I. Slobin (University of California, Berkeley), who spoke on his own work and that of F. Antinucci (CNR, Rome) on children speaking Italian, Serbo-Croatian and Turkish. In general, the syntactic errors common to all children can be traced to cognitive problems inherent in what they have to express. Purely linguistic difficulties vary between languages so that, for example, Yugoslavian children are slower to grasp inflection, which is complex in Serbo-Croatian, but quicker than Turkish children to grasp relative clause structure, which is particularly difficult in Turkish. All this raises implicitly the question Cromer made explicit at the conclusion of his assessment of the field: should language learning be considered separately from other kinds of learning? He did not venture an answer, but on the basis of evidence presented at the symposium it should be no.

A. Buffery (University of London) presented a review of recent anatomical and psychophysical evidence for genetically determined asymmetry in the structure and function of the human brain which was widely regarded as evidence for the innateness and the uniqueness of linguistic abilities. But the anatomical evidence rests on an enlargement of part of the left temporal lobe in about 60% of neonates; and there is psychophysical evidence for functional asymmetry in visuo-spatial as well as linguistic skills. It may be legitimate to conclude that man is innately endowed with some elaboration of the cerebral cortex that enables him to use language, but not that it is qualitatively different from whatever allows him to do arithmetic, for instance.

It is beginning to seem that it may be a mistake to investigate the acquisition of language *per se*; and it should perhaps not be very surprising that philosophical linguistics have in the end relatively little to contribute. For one thing, language as spoken to and by children is very different from the sophisticated instrument it becomes in the hands of philosophical linguists. Roger Brown's frequently quoted point, that it was unlikely that a child could ever learn to talk from listening to the speakers at a learned conference, was

well taken, and several speakers addressed themselves to the question of how adults (and especially mothers) adjust their speech to talk to children.

Perhaps one of the most striking differences between children's speech and that of adult academics is that while the child's speech is generally strictly functional, the academic's is often counter-functional. Children thus use their limited lexical and syntactic resources to make their meaning clear, whereas academics may devote all the riches of theirs to obscuring it. Examples were not absent from the papers delivered at the symposium. □

Cosmic rays at Munich

from a Correspondent

The 14th International Cosmic Ray Conference was held at Munich on August 15–29.

A CLAIM for the detection of a new particle or interaction process seems to have become a regular feature at successive biennial cosmic ray conferences. This year it was the turn of the Monopole. In a specially arranged lecture, P. B. Price (University of California, Berkeley) presented the facts to a sceptical audience of some 500 cosmic ray physicists. The evidence is the track of a particle passing through a balloon-borne stack of Cerenkov film, nuclear emulsion and lexan sheets. The researchers (P. B. Price, E. K. Shirk, W. Z. Osborne and L. S. Pirskey) argue that in a stack of 33 lexan sheets the particle produced a track expected of either a nucleus with $125 \leq Z \leq 137$ and velocity $\geq 0.92c$ or a magnetic monopole with a magnetic charge $= 137e$. The Cerenkov/emulsion data indicated that the particle was moving downwards with $v/c = 0.5 \pm_{0.1}^{0.15}$ and was either a nucleus with $Z \sim 80$ or a monopole of charge $137e$. The only common explanation is hence the monopole. The evidence looked good to a critical audience.

Subsequent discussions, both in and out of session, produced one major attack on Price's conclusion. This came from P. H. Fowler (University of Bristol) who argued an alternative explanation in terms of a nucleus of charge ~ 82 interacting twice in the lexan stack and losing three units of charge at each interaction. By the end of the fortnight it was difficult to find many people who were highly convinced by Price's arguments. The most popular attitude was to wait and see.

The cosmic ray field today covers a vast range of topics 'from quasars to

quarks' and this was reflected in the more than 800 original papers presented at the conference.

On the more traditional cosmic ray stage Price and Shirk also presented data on the distribution of heavy nuclei ($Z > 65$) in cosmic rays obtained from a 1.3 m^2 lexan detector aboard Skylab. These researchers have obtained data with very high charge resolution and conclude that the measured charge distribution in the vicinity of the Earth at $\sim 0.5 \text{ GeV}$ per nucleon strongly favours synthesis of these cosmic rays by the r-process (rapid neutron capture) within the last 10^7 yr.

Measurements of cosmic ray particles at very high energies ($> 10^{17} \text{ eV}$ per nucleus) are made indirectly by means of the extensive air showers (EAS) produced in the atmosphere. Conclusions on the composition of the particles at these energies are still far from definite. The energy spectrum of the primary particles is however, now becoming well established up to 10^{20} eV per nucleus. Further analysis presented by the University of Leeds group, using data obtained at the Haverah Park EAS detector array, gives added weight to the evidence against there being a cut-off in the energy spectrum $\sim 5 \times 10^{19} \text{ eV}$. Such a cut-off has been predicted to arise from the interaction of the microwave background radiation (2.7 K) and the cosmic ray particles. The absence of a cut-off seems to be inconsistent with the universal abundance of both the microwave photons and the high energy cosmic ray particles.

Moreover, strong evidence seems to be accumulating for a distinct anisotropy in the celestial arrival direction of the highest energy cosmic rays ($\sim 10^{19} \text{ eV}$). As A. A. Watson (University of Leeds) emphasised, the two highest energy events recorded at Haverah Park ($> 10^{20} \text{ eV}$) both come from directions close to the North Galactic Pole—strongly indicating a non-Galactic origin. Arrival direction analysis of 'muon-rich' EAS (Nottingham University group) gives indication that heavy cosmic ray primaries at lower energies (10^{17} – 10^{18} eV) may also show anisotropy in a similar direction.

From both interest and necessity cosmic ray physicists have to concern themselves with high energy nuclear physics as well as astrophysics. Previous claims for the detection of tachyons and mandelas (heavy, long interaction length particles) in cosmic rays have unfortunately had to be withdrawn at this conference. Moreover none of the recent searches for fractionally charged quarks amongst the secondaries of cosmic rays have proved positive. Other new phenomena still do seem to be present at these energies, however, most notably the X particles discovered

by the Japanese emulsion group. Also it seems to be increasingly difficult to reconcile EAS data with the sealing model of nuclear interactions. □

The breakdown of physics?

from Malcolm MacCallum

A Relativity Workshop was held at Gregynog Hall of the University of Wales on September 1–3.

"God not only plays dice. He also sometimes throws the dice where they cannot be seen." This statement is made by S. W. Hawking (Cambridge University) in his recent work on black holes. About eighteen months ago he demonstrated that quantum processes could lead to the emission of thermal radiation from black holes, so evaporating them. This showed that the formal analogies between entropy and the area of the hole, and temperature and the surface gravity of the hole, are genuine identities. Now Hawking has circulated two preprints which develop this argument, which were discussed along with related papers at the workshop.

In the first he discusses the thermodynamics of black holes. He interprets the meaning of the entropy in terms of lost information about the initial state, proves that the second law of thermodynamics holds, and proceeds to argue, by considering a black hole in a box, that as a consequence of time-reversibility and ergodicity the emission from white holes (the time-reverses of black holes) must be thermal, and that hence black and white holes are indistinguishable.

In the second—"Fundamental Breakdown of Physics in Gravitational Collapse"—Hawking proves that the radiation has thermal statistics as well as spectrum, in agreement with independent work of L. Parker (University of Wisconsin) and R. M. Wald (University of Chicago). He argues that the random loss of information into the black hole implies the breakdown of S-matrix theory and that a density matrix formulation is all one can give. By considering further the white hole in a box he infers a 'Randomicity Principle' that singularities emit all possible configurations with equal probabilities, as pithily described above. Hawking asserts that this is necessitated by CPT invariance or by the non-existence of perpetual motion machines.

B. F. Schutz (University College, Cardiff), the organiser, opening the conference, pointed out that these two papers re-established the primacy of the thermodynamics. Various speakers emphasised the heavy dependence of

Hawking's arguments on the full applicability of standard thermodynamic results such as the meaning of entropy and the ergodicity theorem, and questioned the correctness of these assumptions. Schutz pointed out that application of Hawking's black hole calculations to white holes led to a burst of radiation with Rayleigh-Jeans spectrum at infinite temperature, resulting from particle creation from an initial vacuum. This might upset the thermal nature of any emission from within the hole itself. Schutz suggested some ways out of this difficulty, including the existence of a remnant black hole after the white hole explosion. C. J. S. Clarke (University of York) similarly suggested that a black hole evaporation must leave behind a naked singularity.

M. Perry (Cambridge University) presented some work on the modification of Hawking's picture to allow for strong interactions between the particles produced at high temperatures. Unless a very soft equation of state is used, the only consistent picture is that this is unimportant.

R. Penrose (Oxford University) expressed disquiet with the idea of black holes in boxes re-forming by the time-reverse of the evaporation process, which is important to Hawking's argument about equivalence with white holes. He also offered a modification of Hawking's proposed fluctuation mechanism for temporary destruction of the black hole, and various participants did back-of-envelope calculations to substantiate his point.

S. Fulling (King's College, London) and P. Candelas (Oxford University) gave talks on the technical aspects of quantum field theory in curved space-time, focusing on the definitions of the creation and annihilation operators and the vacuum state, and on the Feynman propagator. These talks explored, *inter alia*, the fact that incautious use of Rindler coordinates in flat space produces results inequivalent to the usual quantisation. Fulling showed that the treatment of the Hawking mechanism due to D. G. Boulware (University of Washington), which predicted no radiation, has failings of this kind.

The meeting was highly informal and many short contributions were made in discussion. The consensus seemed to be that the broad sweep of Hawking's ideas involved a great many interesting points, whose further examination was necessary. This will be an extensive programme since conceptual problems in general relativity, in thermodynamics and in quantum theory and their relation with general relativity, as well as difficult calculations, will be involved. The meeting helped greatly in clarifying some of the questions to be studied. □

review article

Contacts with semi-insulators

H. K. Henisch* & C. Popescu†

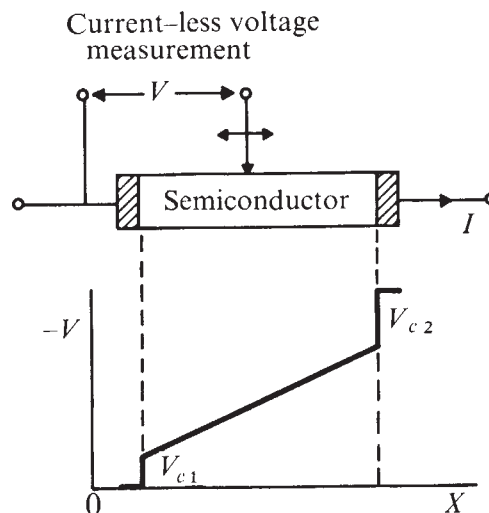
Semiconductor contacts are of considerable practical interest because of their uses in rectification and photovoltaic energy conversion. Recently, however, they have become the focus of the critical controversy because of some unexpected behaviour on the part of contacts associated with semi-insulators.

SEMICONDUCTORS are believed to owe their very discovery to the observation of contact effects in the 1830s. The subject of semiconductor contacts has been of considerable interest ever since, often because contact properties themselves can be very useful, for example in the contexts of rectification and photovoltaic energy conversion¹. In such cases one aims to separate the contact properties from bulk (volume) properties, and to suppress the latter. At other times, when it is the bulk properties that one wishes to select, it turns out that most of these are experimentally "inaccessible" without the use of contacts in one form or another. The suppression of contact effects then becomes the principal aim but this is not necessarily a simple matter, nor indeed is there complete clarity on the criteria for success. Thus, whether wanted or unwanted, contacts (which must be regarded as abrupt heterojunctions) deserve attention and study. They have received a great deal of both in the past, chiefly in the context of technologically important semiconductors such as Ge and Si. Much less is known about effects associated with contacts to semi-insulators, that is materials with resistivities of the order of (say) 10^4 – 10^{10} Ω cm. This is now an active field of study in the context of which new insights have recently been gained.

All precise knowledge of contact effects begins, in principle, with an experiment of the kind shown in Fig. 1. By means of current-less potential probes, whether fixed or movable, the potential drop along a semiconducting bar is explored and compared with the potential difference across the two-terminal system as a whole. This procedure reveals the existence of voltage drops 'at' the contacts, which depend asymmetrically on the current density, as shown in Fig. 2. When this was discovered (on semiconducting sulphides) in the 1870s, it was realised that voltage drops cannot really occur at the interface planes between contacting materials, but must be across some regions of the specimen. Foreign contaminants suggested themselves as a source of the additional resistance, but none could be compellingly identified in that role. It became clear that if some "special region" were involved, it had to be made up of the parent material itself, though modified in some way to make it near-insulating. The explanation of the mystery came with the development of the Mott-Schottky potential barrier models of the 1938–42 period^{2,3}. These models explained not only the voltage drop at contacts but also the asymmetrical departures from Ohm's law associated with them (Fig. 2). These departures result from the presence of carrier concentration gradients which, in turn, imply diffusion currents, to which Ohm's law is in no way applicable. The departures from linearity

are asymmetrical, because the contact barrier is asymmetrically distorted by externally applied voltages, as shown schematically in Fig. 3. Typical barrier thicknesses range from 10^{-6} to 10^{-4} cm, depending on the nature of the surface and the purity of the semiconductor. For an appropriately simplified single-carrier system the carrier transport equation was easily solved, and a voltage-current relationship calculated. Refinements were later introduced to take account of image forces and hot carrier effects, but the model is still used today substantially in its original form. Whether it should be so used is another matter. Schottky himself pointed out that the barrier actually contains too few discrete (and, by assumption, randomly distributed) centres for the space charge to be treated as continuous, and an appropriate correction for this fact has never been devised. Moreover, the model assumes barrier heights greatly in excess of kT and thus applies only to high barriers. Similarly, its validity is limited to thick barriers, for which tunnelling processes can be ruled out. The last two assumptions limit the validity of the model to barriers of high resistance, and whereas no limitation is actually welcome, this particular one is less damaging than one might think because, in practice, we know so much more about contacts of high than of low resistance. This is so because very high voltages are not ordinarily applied to low resistance contacts, and under low voltages (that is voltages smaller than kT/e) all contacts behave almost linearly, as is obvious from Fig. 2. Such contacts may appear "ohmic" for practical purposes, but this does not mean that they are free from barriers (or carrier concentration gradients arising from other causes). Barriers may in fact be present, and would undoubtedly show

Fig. 1 Determination of contact voltages.



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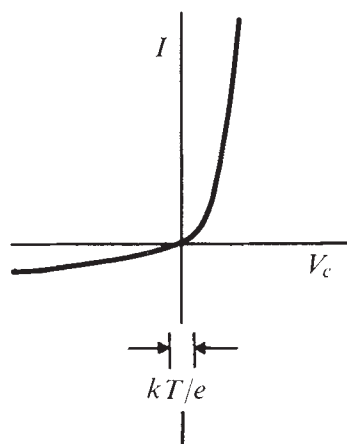


Fig. 2 V - I characteristic of metal-semiconductor contact.

themselves if higher voltages were applied. Such an experiment would, however, have to be carried out with very short voltage pulses, to prevent destruction of the system by heat. Contrary to popular belief, there is no simple relationship between the magnitude of a contact resistance and its linearity. Whether a contact is "ohmic" or not, that is whether it conforms to Ohm's law, thus becomes a relative assessment, and that is actually the only sense in which the term "ohmic" can be safely and unambiguously used.

Because of the experimental difficulties involved, low resistance (low barrier) contacts are rarely studied in a quantitative way. Moreover, they cannot be theoretically handled by the classical transport theory, but call for wave mechanical treatments along the lines of the Fowler-Nordheim analysis, modified by space charge considerations. Such treatments had been completely lacking, until the work of Gossick^{4,5}. "High" and "low" barriers are obviously limiting abstractions as, indeed, are "thick" and "thin" barriers, especially when we are dealing with non-rectangular energy contours of variable shape. The most general case is therefore highly complex and awaits the attention of some dedicated worker with an unflagging enthusiasm for modern computer techniques.

The above account assumes that contact and bulk resistances can always be clearly distinguished from one another, for example by an experiment of the kind shown in Fig. 1. This is equivalent to the assumption that the semiconductor material under a contact remains electronically unchanged by the presence of the current. It was precisely the breakdown of this assumption that led to the invention of the transistor. Bardeen and Bratton⁶ were exploring the field pattern in the neighbourhood of a point contact which was passing a current in the forward direction. They found that the material to a substantial depth under the contact behaved as if it had higher than normal

bulk conductivity. Additional charge carriers were evidently present in the affected region, but charge carriers of one sign could not be contemplated, because the resulting space charges would have been enormous. The effect could not, therefore, be explained on the basis of a single-carrier model, and in response to this difficulty the inventors of the transistor formulated the concept of minority carrier injection. The contention (since then amply confirmed) was that, at the contact and immediately below, the current is carried not by the majority carriers which predominate in the bulk, but by minority carriers. In n -type material, the injected carriers would thus be holes, present in (non-equilibrium) excess concentrations ΔP . These carriers drift and diffuse in the semiconductor as far as their "lifetime" will allow. Of course, their contribution to the current cannot actually "disappear"; in due course injected minority carriers recombine with resident majority carriers in a manner which ensures current continuity. Before the minority carriers have time to recombine, they attract (in media of relatively high conductivity) an excess ΔN of charge-compensating majority carriers into the injection region. The result is an excess of both types of carriers in a region which remains essentially neutral, and this accounts for the higher than normal conductivity. Figure 5a shows this situation. The magnitude of the increments ΔN and ΔP depends on the carrier lifetime, on the height of the contact barrier (relative to the band gap), and on the current density.

In due course it was found that minority carrier injection is only one out of four possible non-equilibrium processes which can be sustained by currents through semiconductor contacts, and are controlled by minority carriers. The others are minority carrier accumulation, exclusion (terms originally coined by P. C. Banbury) and extraction (see Fig. 4). For reasons which are outside the scope of this discussion, only one of these four effects, namely injection, has so far been shown to lend itself to practical applications, and that is why we have (in that sense) only one type of transistor. For the same reason, minority carrier injection is the only process considered here. In specific contexts, the non-equilibrium effects can be negligible, but that a contact should exhibit none of the four effects in greater or lesser degree is actually inconceivable. In the presence of a current, any discontinuity in the conductive properties must lead to the formation of a transition region, within which the carrier concentrations depart from equilibrium, and the current composition adjusts itself to the normal bulk mode. Thus, every contact is associated with contact effects, and we have learned a good deal (if not actually enough) about the design of contacts with specific properties.

Until recently, it was always assumed that the above non-equilibrium effects would be totally suppressed in materials of very short lifetime, but even this apparently self-evident expectation involves an internal conflict. Implicit in the above account is the assumption that the carrier lifetime τ_0 in the semiconductor is much greater than the dielectric relaxation time τ_D ("lifetime semiconductor"). It is this assumption which permits

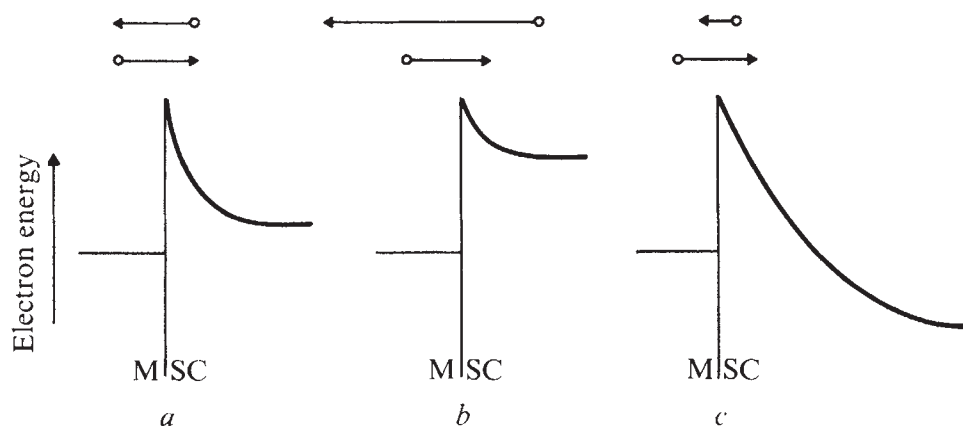


Fig. 3 Rectification through asymmetric distortion of the contact barrier (shown for n -type material). *a*, In equilibrium; *b*, with a forward voltage applied; *c*, with a reverse voltage applied. Arrows denote electron flow. M, metal; SC, semiconductor.

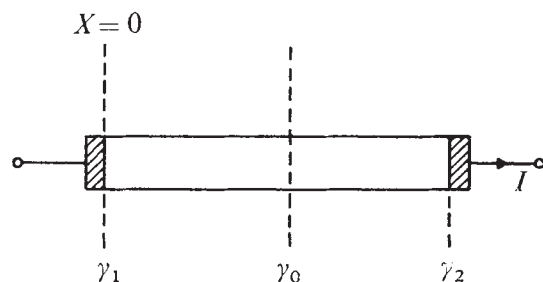


Fig. 4 The four non-equilibrium effects which can result from current flow through a contact. n-Type semiconductor $\gamma_1 > \gamma_0$ injection; $\gamma_1 < \gamma_0$ exclusion; $\gamma_2 > \gamma_0$ extraction; $\gamma_2 < \gamma_0$ accumulation, where γ_0 is current composition rate. γ_0 (its value far from contacts) = I_p/I , where I_p = current carried by minority carriers.

minority carrier space charges to be neutralised by an influx of majority carriers, before carriers recombine. As long as $\tau_D \ll \tau_0$, the supply of compensating charges is always plentiful. If the lifetime τ_0 were to approach zero, however, this inequality could not be maintained, even in the relatively well conducting transistor materials. In materials of higher resistivity the situation is generally reversed, that is $\tau_D \gg \tau_0$, and neutralisation becomes very difficult.

The changes which the assumption $\tau_D > \tau_0$ implies in our picture of contact injection were not appreciated until recent years, when attention was drawn to them by van Roosbroeck and Casey⁷⁻⁹. In so doing, they opened a completely new field of inquiry. In particular, van Roosbroeck pointed out that minority carrier injection into a semi-insulator (a "relaxation semiconductor") would lead to majority carrier depletion ($\Delta n < 0$), and not to an excess of both carriers, as it does in the conventional lifetime semiconductor. In turn, the majority carrier depletion was expected to lead to a higher than normal total resistance, again in contrast to the lifetime case in which the total resistance of the system is lowered by injection. It was also predicted that the current-voltage relationship of such a system would be sublinear, and that the depletion region would spread throughout most or all of the available material. This spread was expected to have a peculiar and important consequence: it would invalidate one of the most popular tests for contact effects, namely the proportionality between specimen resistance and specimen thickness (bearing in mind that measurements on semi-insulators cannot be performed in the manner suggested by Fig. 1, but only on two-terminal thin film systems, without potential probes). The model suggested that, in relaxation semiconductors, this proportionality would be essentially

maintained in spite of the presence of minority carrier injection. The resistance determined in this way would, however, be a good deal higher than that calculated from the dimensions and the undisturbed bulk resistivity, and to that extent a totally misleading result could be obtained.

Of course, majority carrier depletion can be expected to have an important role only as long as the material contains a significant number of majority carriers to deplete. In really wide-gap insulators, there are virtually none, and all injection (whether of majority or minority carriers) then gives rise to purely space charge controlled conductors processes, of the kind analysed and discussed by Lampert and Mark⁹. These processes give rise to a proportionality between (current) and (voltage)², equivalent to Child's law in a vacuum, but different because of the limited mean free path of carriers in a solid. In what follows, we are concerned with poorly conducting materials in which, however, resident carriers still play an important part.

The startling van Roosbroeck predictions were duly challenged in the literature, and even the notion of majority carrier depletion as such was thrown into serious doubt. One of the difficulties is that the two-carrier transport equations cannot be explicitly solved. "Simplifying assumptions" can certainly be introduced, but whether these distort the picture or are truly helpful can be reliably assessed only by comparison with the full solutions, and these must come from the computer. They have only recently become available (refs 10 and 11 and C.P. and H.K.H., unpublished), and their general significance may be outlined as follows.

The relationships can be best understood by comparison with the more familiar lifetime case. Figure 5 gives computed contours for the additional carrier concentrations ΔP and ΔN , as well as the field, as a function of distance from the injecting interface. Figure 5a and b are for the same normalised current density, the only difference being the ratio τ_D/τ_0 (or, somewhat more accurately, τ_{Dn}/τ_0 , where τ_{Dn} is the relaxation time arising from majority carriers alone). It will be seen that the majority carriers are indeed strongly depleted (ΔN negative) under the contact in the relaxation case, as van Roosbroeck had suggested. The disturbance extends over a number of Debye lengths which, in a semi-insulator, can amount to an appreciable depth. The depletion is virtually complete and, accordingly, the recombination of injected minority carriers is inhibited in this region. It therefore takes place in "recombination front" (where ΔN has its sharp gradient). It can be shown that the total carrier concentration $N+P$ is also depleted over a certain distance and has a well defined minimum. This minimum falls, however, within a region in which the diffusion currents are high, and in such a region carrier concentrations do not control the current; concentration gradients do. The minimum of $N+P$

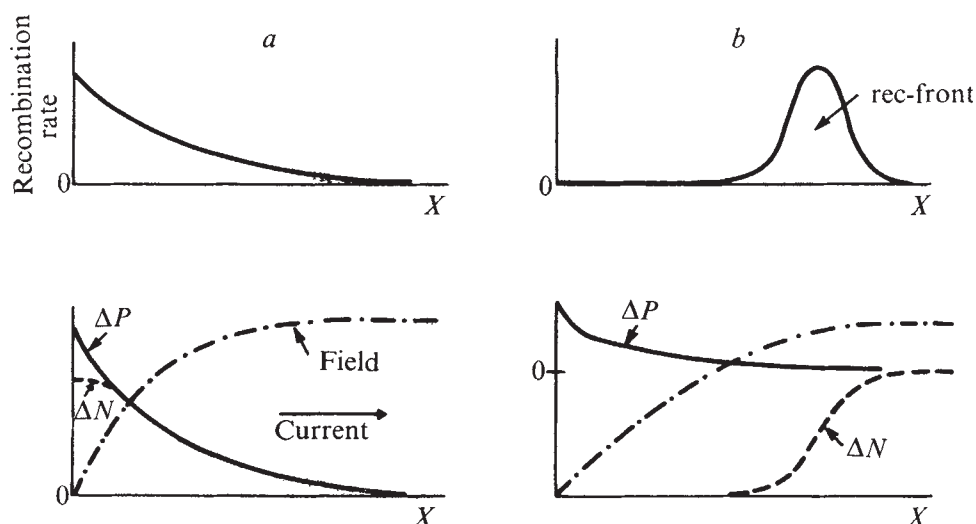


Fig. 5 Consequences of minority carrier injection into n-type material. a, Lifetime semiconductor ($\tau_D/\tau_0 \leq 1$); b, relaxation semiconductor ($\tau_{Dn}/\tau_0 \geq 1$), for same (normalised) current density.

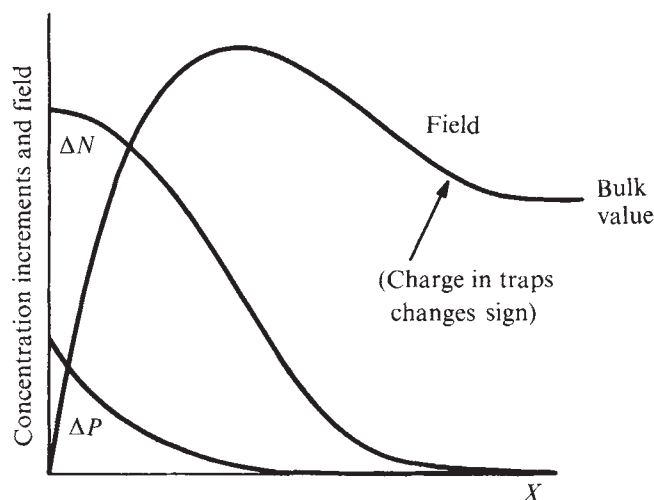


Fig. 6 Carrier concentration and field contours for minority carrier injection into a relaxation semiconductor containing traps.

does not, therefore, govern the resistance of the system as a whole. Assuming bimolecular recombination and equal electron and hole mobilities, the total resistance can be shown to be smaller than that expected from the bulk resistivity, in spite of the majority carrier depletion. The presence of diffusion currents contributes to this fact, but the physical picture is clear enough even without them. The resistance is actually determined by two opposing tendencies, of which the first wins: (1) the high concentration of injected minority carriers, and (2) the depletion of resident majority carriers.

It is interesting to consider the behaviour of such an injection system under illumination¹¹. In the presence of radiation, the space charge system is modified; accordingly, recovery (when the radiation ceases) involves not only the recombination of excess carriers but a space charge movement. This means that the time constant of photoconductive decay is not a pure carrier lifetime, but a complex mixture of recombination and relaxation effects, involving τ_D as well as τ_0 . As a consequence, carrier lifetimes cannot ordinarily be determined from photoconductive decay experiments in the relaxation case. The calculations of Fig. 5 refer explicitly to the contacts of "unit injection ratio", that is contacts for which the entire current is made up of minority carriers at the metal-semiconductor interface. In terms of a semi-infinite n-type semiconductor, this means $J_0 = J_{p0} = J_\infty$, where J_∞ , the current far from the contact, is, of course, carried by electrons. Quantitatively, the available results are limited to their case, but *mutatis mutandis*, the model applies also to lower injection ratios. Of course, all injection effects are then diminished.

If the above (idealised) model yields only lower than normal resistances, as it does, there remains the interesting question of whether higher than normal resistances can ever be envisaged, as originally suggested by van Roosbroeck. A search for such forms of behaviour must inevitably turn to systems of a more realistic (albeit more complicated) kind. To be realistic, a model must include some consideration of trapping processes, which are almost universally characteristic of semi-insulating and insulating materials. Trapping has two aspects: (1) it involves recombination through centres, implying a carrier lifetime which is not constant, as assumed above, but dependent on injection level, and (2) it involves space charge storage. Of these, the second is actually the more potent factor, as recent calculations (C.P. and H.K.H., unpublished) have shown. To understand the role of the traps, it is convenient to refer again to Fig. 5a, even though these particular contours apply to trap-free conditions in a normal lifetime semiconductor. It will be seen that over most of the injection region neutrality prevails, but close to $X = 0$ there is an imbalance, which creates an electric

field. This field actually serves two distinct purposes, both designed to provide electrons in sufficient numbers to satisfy the recombination needs: (1) the field supports electron drift towards the injecting plane, and (2) it opposes electron diffusion under the prevailing (negative) concentration gradient. Because of (2), the field is locally greater than that required to sustain the electron recombination current as such. The greater the concentration gradient, the greater would the field in this region have to be. These relationships are general, but they have a particularly important bearing on the case with traps.

In n-type material trapping states (assumed for simplicity to be located half way across the forbidden band and in equal communication with electrons and holes), would change their occupancy in response to any non-equilibrium situation; in other words, these traps could trap electrons or holes, depending on the relative concentrations. It is then an easy matter to show, on the basis of quite conventional Shockley-Read considerations, that the trap response to excess holes is much more sensitive than that to excess electrons; the traps are overwhelmingly hole traps (as long as there are holes to trap). A very small unbalance in the equilibrium trap occupancy is sufficient to produce enormous fields, and if there were no compensating mechanism, the positive space charge density would soon become quite excessive for the maintenance of current continuity. There is, however, a compensating mechanism, namely the attraction of majority carriers from the bulk material (Fig. 6). Accordingly, an excess ΔN of free electrons appears in the injection region. We then have $\Delta N > \Delta P$, the charge difference being made up (almost everywhere except close to $X = 0$) by the trapped (immobile) holes. This happens in the relaxation case as well as the lifetime case. Indeed the presence of traps can change the situation from a relaxation regime to a lifetime regime. The appropriate criterion is then no longer τ_{Dn}/τ_0 , but $\tau_{Dn}/\tau_0 (M_0 + 1)$, where M_0 is the (normalised) trap concentration. In the trap-free case, the field has its highest value in the bulk, as Fig. 5 shows, but when traps are present in sufficient concentration, the maximum field is in the disturbed region (Fig. 6). This means that the total resistance is indeed higher than that expected from the bulk resistivity, though for reasons very different than those originally envisaged by relaxation semiconductor models. The emphasis here is not on the fact that we are dealing with injection into a relaxation semiconductor but on the fact that we are dealing with injection into a material containing large numbers of traps. That is when the negative gradient of majority carriers appears, even when the carrier lifetime is shorter than the relaxation time. In otherwise comparable conditions, lifetime semiconductors can be shown to exhibit similar and, indeed, more pronounced resistance enhancement effects. In Fig. 6, the field is at its maximum necessarily where the concentration gradient is at its maximum (and not within a majority carrier depletion region). The general conditions of current continuity are maintained. Of course, high carrier lifetime coupled with high trap density do not ordinarily go together, but in good photoconductors they do. Moreover, recent observations on gallium arsenide along these lines by Ilegems and Queisser of Bell Telephone Laboratories may refer to just such a combination of parameters, and the internal resistance of many junction-type solar cells must depend on these considerations.

One of the interesting aspects of the matter is the way in which a challenge of long cherished assumptions has led to completely unforeseen forms of behaviour. Moreover, what has been done so far is only a beginning; virtually every kind of electric, photoelectric and galvanomagnetic measurement procedure applied to semi-insulators is due for re-examination, a process which will no doubt take several years, and which may lead to new applications as well as a new understanding of transport processes.

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articles

Limits to energy release and utilisation from chemical fuels

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New combustors effectively abolish limits of flammability and extend the concept of what is a fuel. By burning very lean mixtures they also minimise pollution and irretrievable energy losses during conversion. The theoretical saving is more than double the proportion currently contributed by all nuclear and other 'non-combustion' sources.

PREVIOUS communications^{1,2} re-examined combustion strategies in response to energy shortages and described one particular burner³ for mixtures of very low heat content. This was based on massive heat recirculation between products and reactants, without simultaneous dilution by recycling products. Quite small models enabled mixtures to be burnt which corresponded in heat content to only one-third of the normal "limit of flammability" and this at flow rates two orders of magnitude in excess of the burning velocity of limit mixtures.

In terms of the conventional definition of a fuel as "something that will burn" such devices evidently extend the definition of "what is a fuel?" Quite apart from the various sources of very lean mixtures which could be burned by such methods³, (for example, ventilation air from mines, waste gases from various industrial and fermentation processes) many materials potentially capable of exothermic reaction are not now regarded as fuels because they contain a large proportion of inerts. Although brandy is generally recognised as a fuel in a combustion as well as a metabolic sense, beer, for example, would probably be considered so only in the latter sense. More seriously, methane diluted with 90% of inert gases would not previously have been rated as a fuel because it would not burn in air—that is, the stoichiometric mixture it would produce with air, if it were to burn as a diffusion flame, would lie below the normal limit of flammability.

In the majority of applications the question is not only "what can we burn?" but also "how much of the energy released can we usefully abstract?" There are, in fact, quite a few exceptions to this in the realm of the combustion of very lean mixtures because it is often desirable simply to burn up noxious pollutants to more innocuous products. In the present—and future—climate of energy shortages, however, the thermodynamic potential at which combustion energy can be utilised must always be a major consideration.

Since an improvement of energy utilisation from combustion of only 12.5% could, at present, replace all nuclear and other energy sources, we feel, generally, that insufficient attention is being paid to these problems. Accordingly, we have investigated

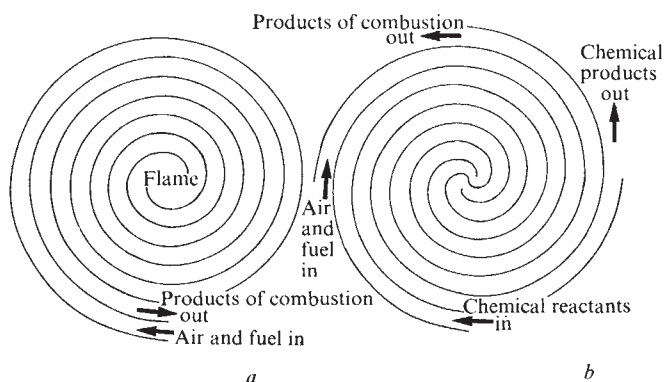
the absolute minimum heat contents at which stable burning can be maintained using the principle of recirculatory burning and the thermodynamics of conversion of the heat released from such lean mixtures.

Minimum heat content for combustion

The geometry of the "Swiss roll" burner³ is most readily appreciated by reference to a diagram of its cross-section (Fig. 1a). This is purely schematic: the depth (perpendicular to the diagram), the width and number of turns, the size, nature, and packing of the central combustion chamber, as well as the material of construction, are all variable. Since the maximum temperature attained in a combustion zone (see Fig. 2a) is determined by the amount of heat transfer, the reaction rate and thus the heat content of the 'minimum combustible' mixture are all functions of these geometric variables.

In the series of experiments designed to establish the leanest mixture which could be burnt, no heat was abstracted. The main sequence of experiments was carried out using methane as fuel, though a few tests were carried out on hydrogen, propane and ethyl alcohol. The burners were constructed of Inconel 600 strip, the width, number of turns of the spiral, and insulation being progressively increased to minimise heat losses as the fuel content was decreased. Thermocouples sealed into the spiral recorded the five temperatures in the positions shown in Fig. 2a; the initial, final, maximum just before and just after the combustor being denoted by subscripts 0, f, m, 1 and 2 respectively. Variation of flow velocity for each set of conditions produced

Fig. 1 Schematic of spiral cross section. a, Simple burner; b, second channel for processing another reactant.



stability loops similar to those observed previously³. If there were no heat losses, first law considerations demand that

$$T_m = T_1 + q = T_2 = T_0 + (T_2 - T_1) + q$$

where q is the temperature rise across the combustion zone, also equal to $(T_1 - T_0)$, and $(T_1 - T_0) = (T_2 - T_1)$. Absence of dissociation at these low temperatures, constant specific heat and a heat of reaction independent of temperature are assumed throughout for these highly diluted mixtures. In the present experiments there were always some heat losses (though it is possible to eliminate them almost completely as we show later) and errors in thermocouple readings include radiation exchange with the walls as well as catalysis, particularly for T_1 , at low fuel concentrations. Labelling heat losses as shown in Fig. 2a, the above equations become:

$$\begin{aligned} T_m &= T_1 + q - L_2 = T_2 + L_3 \\ &= T_0 + (T_2 - T_1) + q - (L_1 + L_2 + L_4) \end{aligned}$$

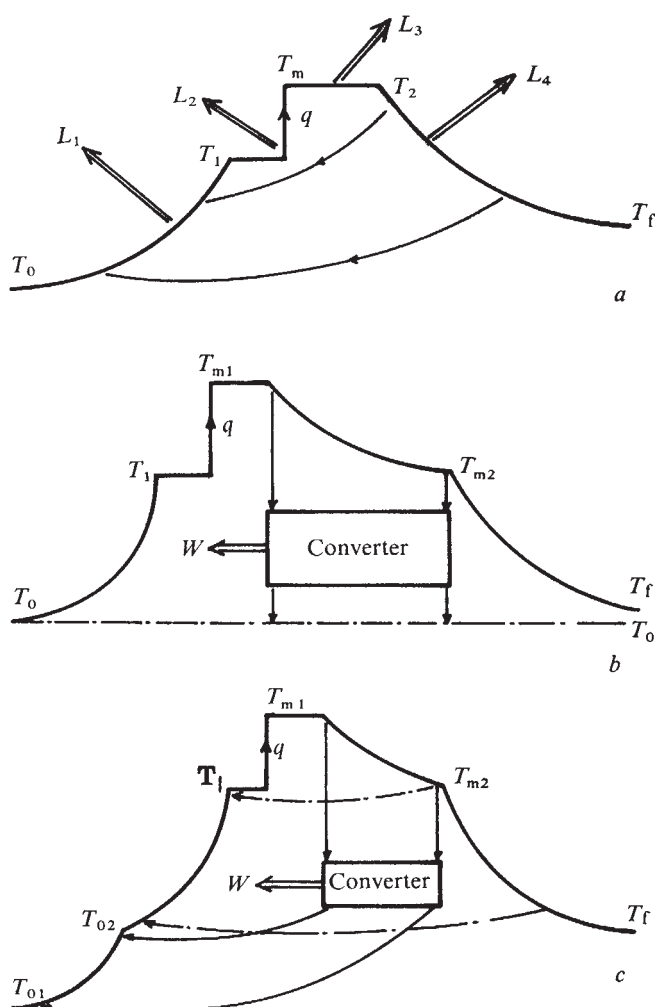
where

$$L_1 + L_2 + L_3 + L_4 = q - T_1 + T_0$$

and

$$L_1 + L_4 = (T_2 - T_1) - (T_1 - T_0)$$

Fig. 2 Temperature schemes without (a) and with (b,c) heat abstraction.



These, together with the measured values, were used to establish criteria governing stability limits.

The richest mixture studied was well below the flammability limit (5.3% methane in air) at 3.7% methane. Increasing the number of turns on the spiral for a given width of strip at first enabled leaner mixtures to be burnt. Plotting the leanest fuel concentration against the number of turns resulted in, approximately, rectangular hyperbolae—the decrease levelling off as sideways losses became controlling. Increasing the width of the strip and adding insulation caused the decrease in limiting fuel concentration to be resumed. We did not continue this progression to much below methane concentrations of 1%.

The salient feature which correlated the various temperature results (to be published in detail elsewhere) was extremely simple: the temperature in the reaction zone at the limit remained constant, within experimental error. For methane, this temperature is approximately 1,400 K. The explanation is thought to be that the limiting reaction rate is constant and, because of the high activation energy, very much more dependent on temperature than on composition. Using an overall activation energy⁷ of 268 kJ mol⁻¹, a change in the reactant concentration from 1.0 to 0.5% would correspond to a temperature rise of only 45 K.

Results obtained by Smith⁴ in a study of the temperature dependence of limits of flammability can be interpreted similarly. Although the mixtures were preheated by an external source and prereaction did not enable him to go leaner than 3.4% methane, the final flame temperature which can be calculated from the sum of his chemical and thermal enthalpies is in fact remarkably close to ours, at 1,430 K. Our much leaner mixtures burn without external intervention at much the same temperature and our extrapolation of this relationship would intersect the 'pure air' axis at about that temperature.

Having gone 82% of the way from the normal limit of flammability towards pure air, we discontinued the experiments and concluded that for all practical purposes there are no limits of flammability for combustion, when heat recirculation is not accompanied by simultaneous dilution of the reactants with products. In practice, sideways heat losses can be abolished altogether by turning the 'Swiss roll' into a torus. For materials which are not sufficiently flexible, or do not pass through a plastic stage during manufacture (as do some ceramics) an assembly of slices with non-parallel sides may be used. Such a configuration is of course not designed to withdraw heat at the highest temperature and, with a sufficient number of turns of the spiral, the product enthalpy flow is the only heat exit. In these conditions, however, anything capable of even marginally exothermic reactions becomes a potential fuel. The final temperature corresponding to the leanest mixture we burnt was approximately 225 K and, on the above activation energy argument, 0.22% methane should be possible before the temperature in the reaction zone would begin to damage our construction material.

Possible applications in which heat abstraction at the maximum temperature is not required include room heating, allowing the product to escape directly into the room. The saturated vapour pressure of conventional fuels is quite sufficient to enable air saturated with the fuel at room temperature to be burnt without previous atomisation or pumping and the combustion temperature ensures both complete combustion and negligible formation of CO and oxides of N₂. For burning up certain industrial waste gases and pollutants, the residual gas temperature may only be sufficient to provide enough buoyancy for dispersal through a stack. Among other proposals is the humidification, heating and sterilisation of hospital atmospheres by injecting a small proportion of hydrogen before passage through the burner.

Thermodynamic efficiency

When considering heat abstraction at the maximum temperature, for example, for conversion into other forms of energy, the conclusions are no less surprising than the finding that such

burners manifest effectively no limits of flammability. The striking conclusion is that, even starting from prime fuels, a higher conversion efficiency can result if the fuel is first diluted with a great excess of air.

There is a great variety of possible schemes for incorporating a converter, or heat engine, into the heat recycle. Figure 2*b* and *c* shows two examples: the former rejecting to an infinite (isothermal) heat sink and the latter to the heat exchanger inlet. A cursory treatment⁶ of the theory is somewhat inadequate and a more detailed account is being published elsewhere⁸. Although the overall efficiency is highest when the system shown in Fig. 2*b* is used with a thermodynamically efficient converter, for inefficient (for example, thermionic) converters it may be advantageous to use a different configuration. One possible reason is the large electrode area made available if parts of the spiral are used in that capacity³. For an inefficient converter, this advantage can outweigh the disadvantage of the rising temperature of the heat sink, particularly where the efficiency is overwhelmingly dependent on the temperature at which electrons are emitted⁵.

In this instance, however, we are concerned with the maximum amount (hence also pumping and other losses are neglected) of energy available. If we postulate converters consisting of a succession of elemental Carnot cycles the efficiencies corresponding to Fig. 2*b* and *c* can be shown⁶ to be

$$1 - [T_0/(T_{m1} - T_{m2})] \ln(T_{m1}/T_{m2})$$

and

$$1 - T_{02}/T_{m1} = 1 - T_{01}/T_{m2}$$

respectively. The nearest practical approach to these idealisations would be represented by two Stirling motors, their 'hot ends' contacting the sides of the combustion chamber in the centre of the 'Swiss Roll', their 'cold ends' being exposed to ambient conditions or, in the case shown in Fig. 2*c*, to a regenerator from which the reactants enter the spiral. The sides of the spiral would be insulated. In these conditions, the maximum value of temperature, T_{m1} , and thus of the efficiency, is entirely limited by the melting point of the confining material used and totally independent of the heat of reaction, since the heat recycle enables any temperature up to that maximum to be reached. (But for this restriction—and the effects of high temperature on dissociation and q —we could attain 100% efficiency.) As T_m tends towards infinity, the heat rejection tends towards zero. If we now allow the converter to use all the heat of reaction—that is, if we make the heat exchanger long enough to transfer the required amount of heat on a temperature differential which is negligible by comparison with that across the reaction zone—the two expressions for efficiency become

$$1 - (T_0/q) \ln[T_m/(T_m - q)] \text{ and } 1 - T_0/(T_m - q).$$

It follows that the efficiency increases as the temperature rise across the combustion zone falls, attaining the Carnot value $1 - T_0/T_m$ as q tends towards zero. The initially surprising result that efficiency can actually be improved by diluting the fuel, and making up the maximum temperature by recirculation, is simply due to the smaller temperature fall during the abstraction of the heat of combustion. The trend to the Carnot value in the first efficiency expression becomes more obvious on writing $\ln[T_m/(T_m - q)]$ as $\ln[1 + q/(T_m - q)]$ and expanding the logarithm for small values of q , whereupon the first expression reduces to the second.

The startling consequences become apparent on introducing realistic numerical values. Taking the maximum temperature at which the converter walls would melt, or oxidise, as the modest value of 1,500 K, the Carnot efficiency to which the above expressions tend at increasing dilution, is 80%. For the leanest mixtures burnt in the present work, $q/(T_m - q) \approx 0.18$. The corresponding efficiencies for the two systems are as high

as 78.3% and 76.5%, respectively. Using a richer mixture—such as would produce a final flame temperature of 1,500 K without recirculation—would give a maximum efficiency (external heat sink) of less than 60%. Thus the gain obtained from dilution and recirculation is equivalent to burning approximately one-third more fuel. On a global scale, this would provide more than double the requirement for replacing all nuclear contributions. Yet it takes no account of the difficulty of stabilising a flame of even that final temperature by conventional means. 1,500 K would be considered a very low flame temperature and it is the need for flame stability which is generally responsible for our using near-stoichiometric mixtures whose product gases, having generated oxides of nitrogen at these high temperatures, then have to be cooled down before they become acceptable to the converter.



Fig. 3 Radiant emission at maximum temperature.

This argument clearly applies only to systems limited by the melting point of walls. Where the hot gas is expanded while confined by cooled surfaces, other limitations, due to quenching, and so on, apply. As 1,500 K already corresponds to a Carnot efficiency of 80%, comparison with efficiencies attained in practice may cause us to reconsider the need for high combustion temperatures.

Similar considerations apply to abstracting heat at the maximum temperature, T_m , for purposes other than energy conversion (as distinct from using only the enthalpy of the product gases, at T_f , which we discussed earlier). For heat transfer by radiation, for example, using the temperature at the top of the 'thermal dam' is essential. Figure 3 illustrates this for a system fitted with a quartz window, the beam being made visible by scattering from smoke introduced in the vicinity and by the use of a 'luminous mantle' in the combustion chamber to increase the visible component of the radiation.

Figure 1*b* shows a four start spiral which was constructed for processing a fluid at the high temperature. Of the two neighbouring channels, one carries the combustion gases as before and the other the gas which, for example, undergoes an endothermic reaction at the high temperature. This may be used for the production of a higher grade fuel from the heat released by a lean mixture. The endothermic water-gas reaction, or reforming, provide simple examples of producing a higher specific calorific value from a low one and retrieving the unused thermal

energy. Although the heat flows are coupled, the mass flow rates in the two streams are of course entirely independent.

In the processing of solids, conveyance along spiral channels would be difficult and a more conventional furnace geometry is obtained by passing the furnace tunnel axially through a deep spiral. So long as heat carried away by the material processed and lost by conduction along the refractory tube is minimised, the essential principle of decoupling the heat flow from that of the product gases (which again leave through the spiral) can be maintained. Solid and liquid fuels which pyrolyse pose a similar problem. In this instance the most versatile arrangement is based on a fluidised bed in the combustion chamber, the surrounding spiral carrying only the incoming air and the departing product gases, the fuel being fed directly into the combustor. We are of course considering very low grade fuels—including various forms of refuse—so that strictures on the use of diffusion flames² do not apply. Quite apart from the limitations imposed on maximum mean temperatures, maximum local temperatures are limited by the high diluent content of the stoichiometric mixtures which occur in the inter-diffusion zones.

Conclusions

Burners and combustion equipment generally have always been exceedingly simple, and given the availability of prime fuels and lack of concern about pollution, there is no reason why they should not have been. We have shown that such a minor escalation in complexity (in comparison with, for example, energy release by nuclear reactions) as the use of massive heat recirculation will greatly widen the limits to the release and utilisation of energy. It effectively abolishes limits of flammability, makes anything that is capable of exothermic reaction into a "fuel" and confines the limitations in energy conversion largely to those of the converter itself.

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Strained mixed-cluster model for glass structure

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Many features of glass behaviour can be explained by a new approach to glass structure based on two ideas. First, glass-forming melts generate 'clusters' of structurally non-related polymorphs which associate on cooling but cannot nucleate, and second, inter-cluster thermal strain can be relieved by subsequent 'plating-out' of modifier impurities.

GLASSES comprise a wide class of solids ranging from the familiar soda-lime-silica window glasses to relatively little known arsenide and phosphide based semiconducting materials¹⁻³. Perhaps the one feature all glasses have in common, and which defines an amorphous material as a glass, is a 'glass temperature', T_g . Above this temperature a glass behaves as a supercooled liquid, with a finite though possibly large viscosity; below T_g a glass is a solid, and, at least at low stresses, behaves elastically. T_g can be somewhat varied by cooling rate, and is associated with discontinuities in the temperature variation of many physical parameters, for example thermal expansion, specific heat and refractive index. Any convincing model of glass should be able to explain such behaviour.

Glasses usually show a number of other features, not all of which are readily understood on the basis of one or other of the generally accepted approaches to understanding glass: the network and microcrystalline models (for description see refs 4-7). Thus, there is evidence from X-ray diffraction studies that the short range order in glasses is similar to that in corresponding crystalline solids⁸⁻¹⁰, a conclusion echoed by Raman and backed up by infrared phonon studies (see, for example, refs 11 and 12). There is also evidence from small-angle X-ray scattering of 'micro-heterogeneities' in glass on the 20-Å scale¹³. Even more strikingly, changes in thermal expansion at temperatures corresponding to solid-state polymorphic transitions in related crystalline phases have been found in some oxide glasses, for example, in vanadium phosphate¹⁴ and even in pure silica (C. F. Drake, personal

communication). Such behaviour is of course easy to understand on the basis of a microcrystalline model of glass. What is less easy to understand in these terms, however, is the effect of adding 'impurities' on the viscosity, workability and recrystallisation characteristics of oxide glasses. This, of course, is the basis of the powerful semiempirical techniques developed in the glass industry for preparing 'good' glass: the complex compositions, with some constituents only at a concentration of about 1%, are not used just because only impure raw materials are available at an economic cost, but because they have a real effect, particularly on devitrification properties. A 'good' glass has a shallow viscosity-temperature characteristic and remains workable over a large range of temperature; it most certainly does not recrystallise. It is here that the network model seems particularly applicable, which may account for its almost universal adoption in the glass industry.

Could these two models be unified in some way to give a more general explanation of the properties of glass? The unconventional but simple approach outlined here does seem to suggest a means of achieving this. One point seems of particular relevance: a surprisingly large number of the systems which form glasses have as a major (sometimes the only) constituent a material that exists in two or more polymorphic forms which only differ slightly in free energy. Silica, as an obvious example, is found as quartz, cristobalite and tridymite (and in a number of other less wellknown forms too), but polymorphism is also found with BeF_2 , PbO , As_2O_3 , TiO_2 , selenium, sulphur and CdP_2 , to name several materials of interest in the context of glass formation. Could this polymorphism be a necessary condition for a material to be a glass former? Consider a melt of such a polymorphic material being slowly cooled. At some temperature, associations of the constituent atoms will grow into 'flicker-clusters', incipient nuclei of all possible polymorphs (any significant difference in free energy, of perhaps more than 5 kT, or about 0.1 eV at 1,000 K, would be expected to reduce greatly the contribution of the phase with higher free energy, although

bulk free energies may only be a rough guide to the situation with such small quasi-crystallites). The existence of flicker-clusters in liquids near their freezing point is well established; their formation for example, is the reason for the maximum density of water occurring at 4 °C (ref. 15).

The behaviour of such a system with two or more distinct types of flicker-cluster does not, however, seem to have been considered previously. I suggest that on progressively lowering the temperature such a 'mix' would not behave like the melt of a solid which has only one modification, and that it would be difficult for the clusters to grow to the critical size for nucleation. Instead bonds between clusters with different structure and at random mutual orientation would, initially, continuously form and break, the tendency for their forming increasing as temperature was reduced. The resulting 'mixed-cluster associations' could, presumably, greatly exceed the critical size for nucleation for a single phase cluster without passing the surface energy determined maximum in free energy beyond which runaway crystallisation would normally occur. (Such a situation would give a melt in which viscosity would be expected to increase markedly as temperature fell.) Given this, a temperature would be reached at which a permanent, continuous, bonded network would be set up between mixed clusters, leaving much of the boundaries of the clusters non-bonded and with the material between them still 'liquid-like', even though the system as a whole would have now 'locked up solid', a rigid rather than a viscous material having come into being. Presumably this situation corresponds to some kind of percolation theory limit. It would of course also correspond to T_g . In addition, it is clear from this picture that slow cooling would give rise to a better accommodated continuous network of this kind than fast cooling, leading to just the behaviour found experimentally with T_g in terms of density, refractive index and so on.

An objection to this could be that if two types of cluster were present, A and B, then A-A and B-B contacts could occur as readily as the A-B type discussed above and would tend to lead to nucleation. The important point is, however, that the occurrence of A-B random bonding would hinder and eventually suppress subsequent A-A or B-B epitaxial contacts, which are what are required to reduce surface free energy.

Consider what happens on still further cooling. The liquid and presumably relatively mobile material would remain within the rigid mixed cluster framework gradually crystallising out, but with progressively more distorted bonding arrangement as cluster-cluster boundaries and voids (due largely to thermal contraction) are formed. There would, however, be a crucial further effect operating at the same time. Since the two (or more) cluster-phases would have different crystal structure symmetries, and would be bonded together with more or less random orientation, there must be thermal contraction mismatch between them. This would give rise to progressively increasing strain on cooling which would tend to increase the free energy of the system, making it increasingly unstable with respect to a more normally crystalline variant. (This term then might be particularly important in driving towards devitrification of a glass even above T_g .) It is here that I would suggest that the liquid material bonding out on to mixed clusters is important. Particularly if impurity atoms of correct size were present ('modifiers'), these would grow on to the cluster surfaces or associate at cluster-cluster boundaries in such a way as to reduce the strain contribution to the free energy and also, presumably, the entropy of the system. This, then, qualitatively explains the importance of small additions of specific impurities in stabilising the glassy state. This mechanism, incidentally, is in marked contrast with one put forward by Demishev¹⁶ who postulated microstresses between "an ordered phase" and "disordered material" due to differences in the thermal expansion coefficients of the two kinds of material, which coincide at T_g .

This phenomenological picture of how a glass forms suggests some interesting questions and predictions:

(1) The existence of liquid material with high atomic mobility

below T_g (albeit on an extremely small scale) should be amenable to direct verification by means of motional narrowing of nuclear magnetic resonance if a suitable isotope were available (¹¹B in borate glasses?), although presumably a sensitive apparatus might be needed since only a small proportion of the material would be expected to be involved.

(2) The mixed-cluster model also suggests that immediately below T_g a glass is very like a liquid-filled zeolite, the liquid being held in a system of small interconnecting channels, and with a very large solid surface in contact with it. Interesting effects of ion exchange and diffusional transport should be possible. This view is consistent with the known and highly important "gettering action" of glass films on the surface of silicon semiconductor devices.

(3) Perhaps on the basis of intuition more than argument, it may be possible in a particularly favourable case of glass-forming material and strain-relieving impurities to obtain a system with a lower free energy than any corresponding combination of macrocrystalline phases, that is a glass might be more stable than crystalline material—an almost heretical suggestion but worth further investigation.

(4) If more than the optimum amount of strain-relieving impurity were present in a glass system, the glass would become less stable, simply because there would be a tendency for a new phase to form in the now relatively thick layer of "extra-cluster material" which compositionally would be different from the clusters. If, however, this itself has suitable polymorphs a new mixed cluster system could arise, and still further addition of impurity could restabilise the system. This may offer a generalised explanation for the not uncommon occurrence of compositional systems which show broad glass-making fields, with varying glass stability, for example in the alkali silicates. The important new point here is that in such systems the glass former can be an intermediate compound rather than having to be an end member.

(5) If, as seems possible, the mechanism of strain relief by "plating out" from residual liquid could be a selective process operating not far from equilibrium, this may lead to an explanation of at least the low temperature behaviour summarised under the heading of "the mixed alkali effect" in silicate glasses¹⁷. The simultaneous presence in the liquid of two different-sized alkali atoms would then be expected to lead to a better packed and strain-relieved structure than either could give separately (for example, spheres of two different sizes can pack to fill space more efficiently than a single size of sphere) and thus a denser, more strongly bonded, higher refractive index glass. Such a structure would also account for the observed reduction in alkali diffusion rate in mixed alkali glasses.

(6) The case of vitreous silica is of particular interest. Detailed radial distribution function analyses by Konner and Karle^{8,9} showed the presence of tridymite-like quasi-crystallites perhaps 20 Å across and gave some indication that cristobalite-like material may also be present. As already noted, work in these laboratories by Drake (C. F. Drake, personal communication), using a sensitive dilatometer of his own design, has indicated changes in the slope of the thermal expansion curve of silica (from a variety of sources) which have been persuasively identified as associated with α - β transitions in quasi-crystallites of cristobalite and quartz. There is evidence, therefore, that vitreous silica contains at least three types of cluster. Now Cohen and Roy^{18,19} found that the density of vitreous silica is affected irreversibly by the application of pressures of more than 2×10^9 Pa. This can be interpreted on the present model as due to quasi-crystallites of the least stable phase, tridymite, going over to a more dense coesite-like structure. (The quartz-coesite transition is near 3×10^9 Pa (ref. 20) for bulk material; for tridymite a somewhat lower transition pressure would be expected.) If so, a Konner and Karle type RDF analysis of such densified material should be very different to that which was found for normal vitreous silica. At a sufficiently high pressure of course all quasi-crystallites would become transformed to rutile-like stishovite²¹ and recrystallise-

tion should occur immediately since no "two-or-more-cluster-phase-system" would be in question. Cohen and Roy^{18,19} in fact mention just such behaviour for GeO₂ glass, which would be expected to transform into a rutile-like structure at a lower pressure than for the case of SiO₂. The role of shear in such transformations²² does, however, require further investigation.

(7) It should be possible to distinguish between amorphous solids and those which might, potentially at least, be stabilised as glasses. Amorphous silicon and germanium, for example, can probably not be stabilised, since there seem to be no alternative crystal structures within a reasonable energy range, judging from the very high pressures and compressions required to bring about any phase transformation²³. It might, however, be argued, if the two-polymorph idea is correct, that quenching a germanium melt at a pressure sufficiently high for the freezing point to lie near the phase boundary between (metallic) tetragonal and (semiconducting) diamond type phases could perhaps give rise to a glass. More interesting possibly, is the case of carbon, where at 1 atm pressure ($\sim 10^5$ Pa) graphite and diamond phases differ in free energy by about 500 cal mol⁻¹ (~ 0.03 eV). This suggests that it may be possible to quench liquid carbon to give a glass. Could this be stabilised by transition metals such as iron? Is there any evidence of high viscosity due to graphite-diamond quasi-crystallite cluster formation in the "solvent catalyst" melts used for diamond synthesis, whose mode of action is still not too well explained?²⁴

It also seems likely that where two polymorphs are closely related in structure (for example wurtzite and zinc blende) and have not only the same coordination but differ solely in stacking (so that epitaxial relationship is possible), then the suppression of nucleation described above would be much less likely to occur. (In this context the findings that cristobalite²⁶ and tridymite (J. H. Konnert and J. Karle, personal communication) do not have the simple structures previously assumed to be zinc blende and wurtzite analogues are highly relevant.) For this reason, then, amorphous III-V and II-VI compounds, much like silicon and germanium, should not form glasses. Some reservation may be needed here in that glasses have been made from the related ternary chalcopyrite compounds CdGeAs₂ and CdGeP₂ (refs 1-3). In these two cases the structures of the melts seem to be very different to that found for the simpler diamond-like solids: they have a much lower coordination number, and the binary compounds CdAs₂ and CdP₂ seem to be the glass forming materials.

(8) There is an interesting parallel between the formation of mixed clusters and the behaviour of an eutectic mixture on cooling. At the eutectic temperature two phases crystallise simultaneously but, usually, on a scale which is orders of magnitude larger than has been discussed so far. If it were possible, however, for example by fast cooling, to induce a mixed cluster type structure, the result should be a glass. This may account for the formation of metallic glasses such as Pd₈₀Au₁₁Si₉ by splat cooling²⁵. Such materials tend to recrystallise near T_g as would be expected from this model.

(9) A particularly interesting possibility is that cluster-cluster interfaces (with their attendant strains and voids) at the surface may be associated with the generation of Griffith cracks, which limit the tensile strength of all glasses. Rindone²⁷ notes that "... microheterogeneities of different composition will have different chemical reactivities, especially with water. For example, a potassium-rich region would react with water much more rapidly than would a sodium-rich region. This could lower the strength considerably if there are local tensile stresses around the microheterogeneity due to thermal expansion differences..."

The strained mixed-cluster model, therefore, offers explanations for a wide range of properties found in glasses and can also be used to predict interesting new possibilities. It is, however, only a descriptive approach and obviously requires detailed development before it could be used in anything but the crudest manner. It also may not be applicable to glasses formed from two- or one-dimensional crystal structures, such as long chain polymers, or, even, As₂S₃. This last material in fact seems to be known only in one crystal structure (even though there is a mention of a red β -As₂S₃ in the old literature²⁸) and so, formally, may be an exception to the rule proposed above.

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Magnetic vibrational optical activity in the resonance Raman spectrum of ferrocytochrome c

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The first observations of magnetic Raman optical activity are reported. Using a field of about 0.7T, Raman circular intensity differentials up to $\pm 4 \times 10^{-3}$ are induced in resonance Raman bands of ferrocytochrome c.

OPTICAL activity associated with molecular vibrations can now be studied through a difference in the intensity of Raman scattering in right and left circularly polarised incident light¹⁻⁴.

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Effects found so far in naturally optically active molecules indicate that the Raman circular intensity differential (CID) is a powerful probe of configuration and conformation. All molecules are rendered optically active by static magnetic fields, and should show Raman CIDs if the magnetic field is parallel to the incident laser beam⁵. Magnetic Raman optical activity has not been observed so far at transparent frequencies, but is reported here in the resonance Raman spectrum of ferrocytochrome *c*, a haem protein, using a modest magnetic field.

There are many problems in the application of conventional vibrational spectroscopy to biological molecules. Infrared radiation is absorbed strongly by water, the ubiquitous biological material, and the complexity of biological molecules can lead to thousands of normal modes of vibration. Raman spectroscopy does not suffer from the first limitation as water does not absorb visible and near ultraviolet wavelengths and scatters them only weakly. The second limitation can be overcome through the resonance Raman technique in which the laser frequency falls within an electronic absorption band, usually localised at a site of biological function such as a haem group, resulting in a tremendous enhancement of the Raman intensities of those few vibrations coupled to the electronic transition⁶.

It would clearly be of interest if CIDs could be observed in the resonance Raman spectra of biological molecules as vibrational optical activity, both natural and magnetic, is expected to be sensitive to the delicate configurational and conformational features that determine biological function. Attempts to observe natural optical activity in the resonance Raman spectra of haem proteins and visual pigments on retinal membranes have not been successful so far, possibly because electron conjugation tends to minimise the chirality by holding the chromophores nearly planar. But as porphyrin rings are very susceptible to magnetic perturbations and show large magnetic, optical rotatory dispersion and magnetic circular dichroism^{7,8}, haem proteins are extremely suitable for the observation of magnetic resonance Raman optical activity. Furthermore, the resonance Raman phenomenon enables the contributions from those electronic transitions most susceptible to magnetic perturbations to be isolated, whereas in transparent regions all electronic transitions contribute to Raman scattering.

The quantity that is measured is a dimensionless CID in the light scattered at 90°:

$$\Delta_a = (I_a^R - I_a^L) / (I_a^R + I_a^L) \quad (1)$$

where I_a^R and I_a^L are the scattered intensities with α -polarisation in, respectively right and left circularly polarised incident light. If z is the direction of the incident beam, I_z and I_x are linearly polarised parallel and perpendicular to the scattering plane, yz (Fig. 1). Δ_z and Δ_x are referred to, respectively, as the depolarised and polarised CIDs.

Magnetic Rayleigh and Raman optical activity originate in interference between the scattered electromagnetic waves whose

Fig. 1 The geometry for polarisation experiments in Raman scattering at 90°. z , Direction of the incident beam; y , direction of the detected beam; R , right circular polarisation; L , left circular polarisation.

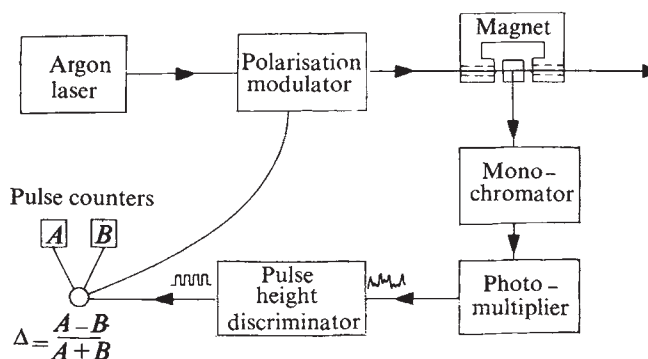
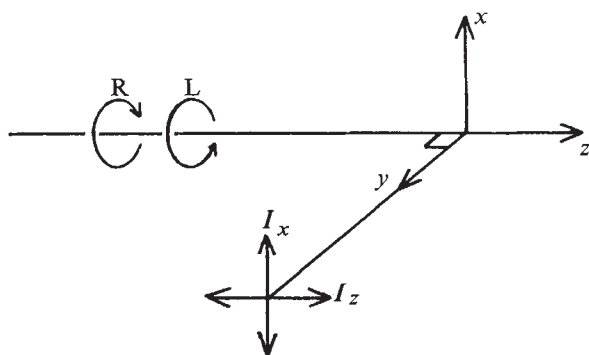


Fig. 2 The experimental arrangement for measuring magnetic Raman CID. A beam from an argon-ion laser is brought to a broad focus in the sample, which is held in a quartz fluorescence cell with a path length of 1 cm. The sample cell is situated between the poles of a permanent magnet with holes bored through the pole pieces so that the magnetic field is parallel to the laser beam. The light scattered at 90° is processed by a triple monochromator and the Raman components detected by a photomultiplier which produces measurable voltage pulses from individual photon strikes. These pass through a discriminator which gives equal weight to all pulses which exceed a certain voltage threshold; this enables much of the noise from the photomultiplier to be eliminated, and gives the scattered intensity as a 'photon' count rate. The polarisation of the incident beam is modulated directly between right and left circular at 9 Hz using a potassium-dihydrogen phosphate crystal driven by a square wave alternating voltage synchronised with two matched pulse counters. While the modulator performs the first half of its cycle and produces right circular polarisation, pulses are channelled into counter *A*; while the modulator performs the second half of its cycle and produces left circular polarisation, pulses are channelled into counter *B*. After accumulating a statistically significant number of counts, the sum of the two counters $A+B = I^R + I^L$ and the difference $A-B = I^R - I^L$ are read. The statistical significance of N counts is determined by the standard deviation $\sqrt{N}$. Thus, on 10^8 counts, the 'statistical noise' is $\sqrt{N}/N = 10^{-4}$, which is an order of magnitude smaller than the larger CIDs expected. Only the depolarised CIDs are sampled (see refs 2 and 4).

amplitudes are proportional to the usual electric dipole-electric dipole polarisability tensor and the same tensor perturbed to first order in the component of the magnetic field along the direction of the incident beam. The basic theory of magnetic Rayleigh and Raman CID was worked out by Barron and Buckingham⁹, although the final expressions apply only to Rayleigh and Raman scattering at transparent frequencies and to Rayleigh scattering at absorbing frequencies. That is because at transparent frequencies, to a good approximation, the real part of the transition polarisability is purely symmetric and the imaginary part is purely antisymmetric; but at resonance this approximation breaks down completely and, in general, the real transition polarisability contains an antisymmetric part and the imaginary transition polarisability contains a symmetric part.

The arrangement for measuring magnetic Raman CID is illustrated in Fig. 2.

One problem anticipated with resonance Raman CID measurements is a bias caused by the circular dichroism of the incident beam. But none was detected to within $\pm 1 \times 10^{-4}$, probably because the magnetic circular dichroism of ferrocytochrome *c* at 5,145 Å is very small⁹. In general, this problem could be overcome by tuning the laser frequency to coincide with the one or more nodes that often occur within a magnetic circular dichroism band.

The depolarised magnetic resonance Raman CID spectrum of ferrocytochrome *c* is shown in Fig. 3. There is good reflection symmetry on reversing the magnetic field direction, and no significant CIDs are found when the field is removed. Magnetic CIDs occur in most of the strong resonance-enhanced Raman bands between 1,100 and 1,600 cm^{-1} . No significant effects are found in the weaker bands in other parts of the spectrum.

These magnetic Raman CIDs involve vibrations of symmetry species B_1 , B_2 and A_2 , assuming an effective symmetry of C_{4v} .

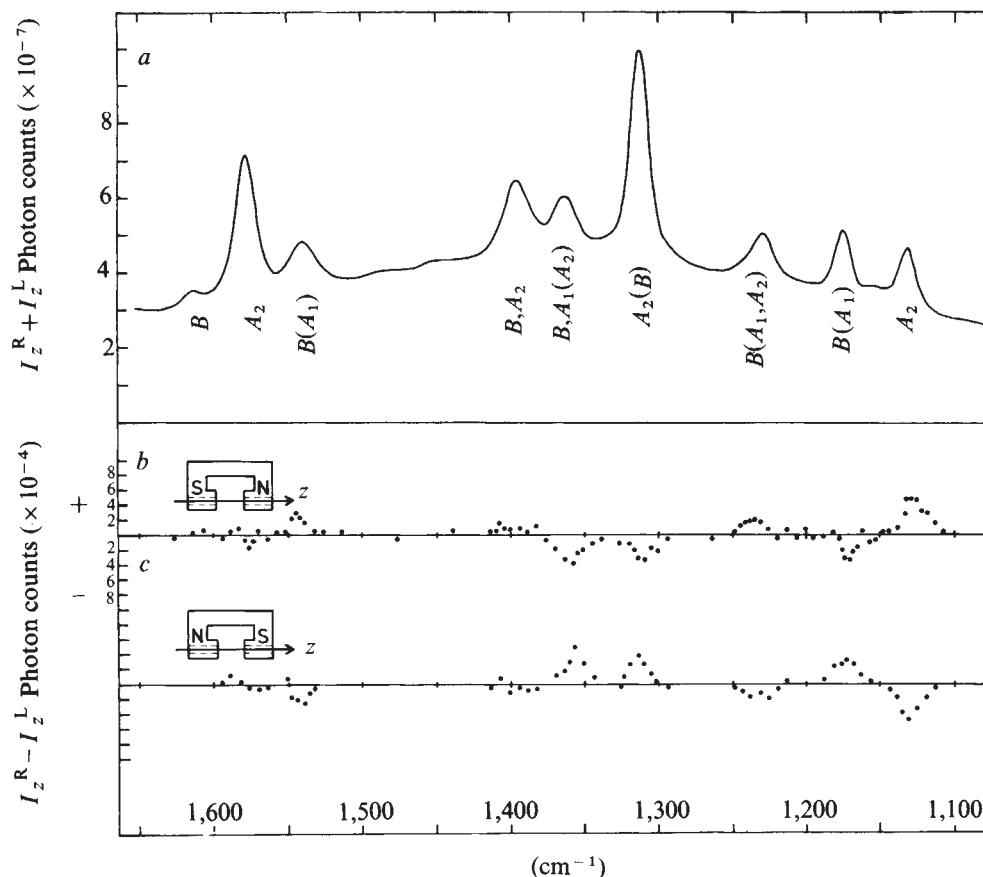


Fig. 3 The depolarised magnetic circular intensity sum (a) and difference (b and c) spectra of ferrocytochrome *c*. The magnetic field directions are opposite in b and c. Experimental conditions: 1×10^{-4} M aqueous solution of cytochrome *c* with excess sodium dithionite; magnetic field strength about 0.7 T; laser wave-

length, 5,145 Å; laser power, 500 mW; slit width, 13 cm⁻¹. The $I_z^R - I_z^L$ spectra were recorded manually at fixed points with a counting period of about 8 min. To estimate the dimensionless CIDs, the background should first be subtracted from the $I_z^R + I_z^L$ spectrum.

for the porphyrin ring in ferrocytochrome *c*, and the symmetry assignments are indicated in the top spectrum. In brackets are shown the minor contributors that arise because the planar tetragonal symmetry of the basic porphyrin ring, which provides a useful first approximation to the classification of the spectroscopic properties, is actually distorted by thioether side groups which link the haem to the protein^{10,11}.

The resonance Raman scattering process involves transitions from the zero vibrational level of the porphyrin ground electronic state to higher vibrational levels of the first π^* excited electronic state ($^1E_u \leftarrow ^1A_{1g}$)¹². Since the ground electronic state is non-degenerate there is no ground state magnetic moment, so the observed magnetic Raman CIDs should be independent of temperature. And as the excited electronic state is doubly degenerate the parts of the magnetically-perturbed transition polarisability that correspond to generalised forms of the Faraday A term of magnetic circular dichroism¹³ should dominate.

A detailed analysis of the magnetic Raman CID spectrum would be premature since the theory is not sufficiently developed and the spectrum is poorly resolved. It is, however, worth noting that the largest magnetic CID ($|\Delta_z| \sim 4 \times 10^{-3}$) is found in the Raman band at about 1,132 cm⁻¹, which originates in a single vibrational mode of A_2 symmetry. Since the corresponding transition polarisability is completely antisymmetric¹⁴, which has so far only been found in resonance Raman bands and is critically dependent on the laser wavelength¹⁵. The other A_2 modes show only weak or negligible magnetic CIDs, and when interpreted these relative CID intensities and their variation with laser wavelength may lead to a better understanding of the

molecular mechanism of antisymmetric resonance Raman scattering, which is still obscure.

The interpretation of the magnetic CID spectrum will be facilitated when examples are found in other molecules, particularly free porphyrins and haemoglobins, in which the chromophore symmetry is better defined so that there is less mixing of the vibrational modes. Unfortunately, it has not yet been possible to obtain results from free porphyrins or haemoglobins since they do not survive long enough in the intense laser beam to allow significant CID measurements to be taken. But with more elaborate sampling arrangements and a stronger magnetic field (an order of magnitude increase is available with superconducting magnets), magnetic Raman optical activity should be accessible in many other samples.

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letters to nature

No indication of excess MeV gamma radiation from the Crab Nebula

DURING the past two years results from two different balloon experiments^{1,2} indicate that the γ -ray emission from the Crab Nebula between 1 and 10 MeV is considerably above the straight line interpolation from lower and higher photon energies. The excess above the power law has been claimed to be factor of 7 between 1 and 3 MeV and of a factor 30 between 3 and 10 MeV. The fact that both experiments yielded nearly identical results gives support to the reality of the excess component. The power in this excess is nearly as large as the total integrated synchrotron power of 2×10^{-7} erg cm⁻² s⁻¹ of the Crab Nebula. It has been speculated that this powerful, narrow-band component may be produced in the pulsar magnetosphere, possibly near the surface of the neutron star².

Results of a balloon flight with our double Compton telescope^{3,4} now set an upper limit to the soft γ -ray flux between 1.5 and 10 MeV from the Crab Nebula which is in severe contradiction to the flux values published previously^{1,2} and rules out the existence of an excess—at least of this magnitude.

The telescope consists of two large plastic scintillator blocks, 1.20 m vertically apart. Both detectors are surrounded by an anticoincidence shield. A γ ray is identified by a first Compton collision in the upper detector and a second in the lower detector—this sequence being confirmed by a time-of-flight measurement. Energy and direction of the γ rays are determined by pulse height measurements in both detectors. The telescope has an acceptance cone of 26° half width at half maximum (HWHM) between 1.5 and 2 MeV, 23° between 2 and 3 MeV, 18° between 3 and 5 MeV and 14° between 5 and 10 MeV. The energy resolution is 40% FWHM at 2.75 MeV. The effective geometrical area for vertical incidence is zero below

1 MeV, increases to 1.6 cm² at 1.5–2 MeV, has a maximum of 2.6 cm² at 3–5 MeV and drops to 2.2 cm² at 5–10 MeV.

The transit of the Crab Nebula was 162 min after the balloon was launched. Because of the large acceptance cone of the telescope the detection probability of γ rays from the Crab drops to half its maximum value ~ 100 min after the transition and is zero ~ 50 min later. In Fig. 1 the total 1.5–10-MeV counting rate is plotted as a function of time after launch. Only those events are taken into account, which satisfy the so called $\bar{\rho} < 30^\circ$ restriction³ and which fall into a certain time-of-flight interval. No statistically significant excess of the counting rate is present over the full observation period. This is also true, if the total energy range is subdivided into four smaller intervals. Although events have also been triggered with energies below 1.5 MeV, these have not been taken into account to avoid uncertainties in the detection efficiency, which are caused at these energies by its strong dependence on the accurate setting of the trigger levels.

For each 20-min interval in Fig. 1 an upper flux limit of the Crab Nebula at the 95% confidence level was determined according to

$$r_{2\sigma} = \int_{1.5}^{10} S(E_\gamma) A \epsilon(E_\gamma, \theta) f_i \exp \left[-\frac{2.5 \text{ g cm}^{-2}}{\lambda(E_\gamma) \cos \theta} \right] dE_\gamma \quad (1)$$

where $r_{2\sigma}$ is the 1.5–10 MeV 2σ counting rate uncertainty in Fig. 1; A is the detector area; $\epsilon(E_\gamma, \theta)$ is the energy and zenith angle dependent detection efficiency; f_i is the fraction of events, for which the time of flight requirement is fulfilled; $\lambda(E_\gamma)$ is the

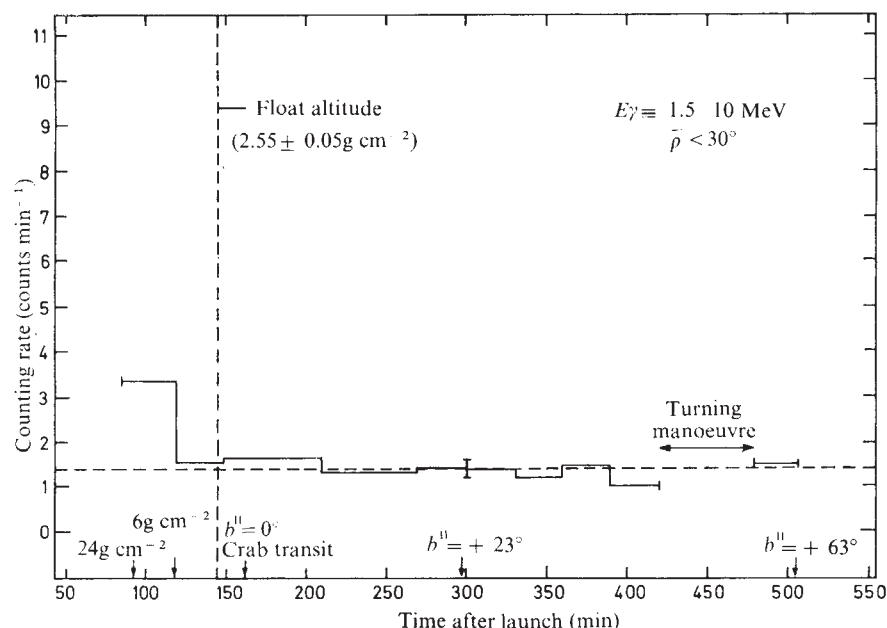


Fig. 1 1.5–10-MeV counting rate of the double Compton telescope as a function of time after launch. Only those events are taken into account for which a certain time of flight requirement and the so-called $\bar{\rho} < 30^\circ$ restriction³ is fulfilled. The balloon was launched from Palestine, Texas on July 11, 1974 at 0905 LT. The balloon reached its float altitude of 2.5 g cm⁻² within 145 min. During the first 4.6 h at float the telescope axis was adjusted to the zenith. Within the next 58 min the axis was rotated from 0 to 360° zenith angle to measure the zenith angle distribution of atmospheric rays. During the last 28 min at float altitude the axis was again adjusted to the zenith. Results from this flight on the diffuse cosmic γ -ray component and on the angular distribution of atmospheric γ -rays have already been published^{5,6}.

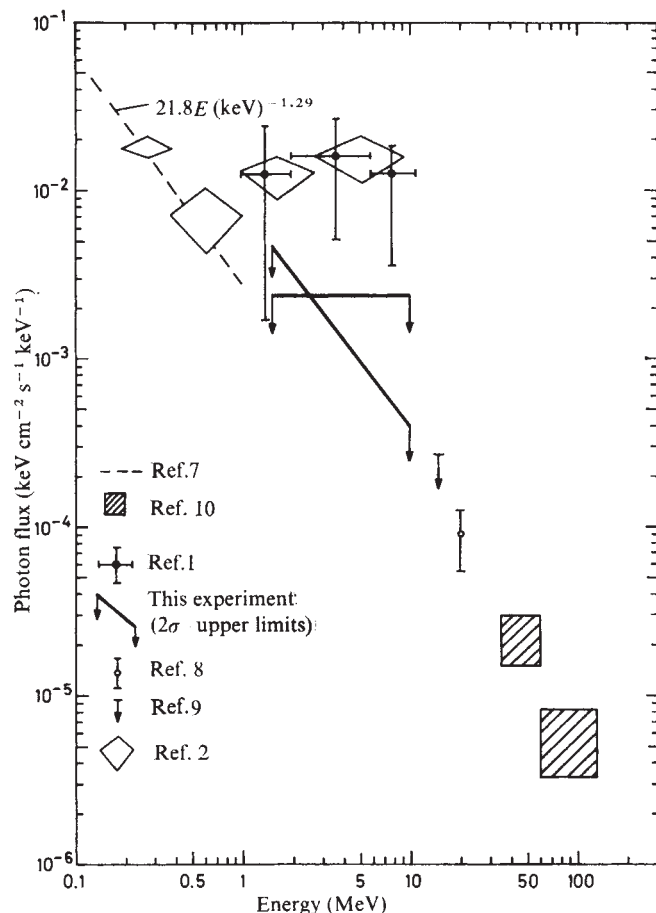


Fig. 2 Energy spectrum of the Crab Nebula. Our upper limits at the 2σ confidence level were derived from the assumption of a power law spectrum between 1.5 and 10 MeV of the form $\sim E^\alpha$ or $\sim E^{-1.3}$.

mean free path of γ rays in the atmosphere; θ is the time-dependent zenith angle of the Crab Nebula; and $S(E_\gamma)dE_\gamma$ is the differential photon number spectrum of the Crab Nebula. For the Crab spectrum a power law with two different exponents has been considered:

$$S(E_\gamma)dE_\gamma = a_1 E_\gamma^{-\alpha_1} dE_\gamma \quad \text{photons cm}^{-2} \text{ s}^{-1} \text{ MeV}^{-1} \quad (2)$$

with $\alpha_1 = 1$ and $\alpha_2 = 2.3$. The first case describes the spectral form as determined by Baker *et al.* and Gruber, the second one is identical with the interpolation from lower and higher photon energies. For each 20-min interval the value for the proportional factor a_1 or a_2 was then determined applying equation (1). The most significant upper limit for the flux of the Crab Nebula is obtained if the limits of each interval from observation periods between 20 min before and 80 min after the transit are averaged quadratically. Then $a_1 = 2.41 \times 10^{-3}$ and $a_2 = 8.24 \times 10^{-3}$. These limits are shown in Fig. 2, where the energy spectrum of the Crab Nebula is shown from X-ray energies to be about 100 MeV.

The upper limit determined from our flight is at least a factor of 5 below the flux values quoted by Baker *et al.*¹ and Gruber² and is only a factor of 3 above the straight line interpolation from lower and higher energies.

If the flux values as determined by Baker *et al.* and Gruber were correct, the total counting rate of our telescope would have been a factor of 3 higher during the transit compared with the counting rate in the second half of our flight. In comparison, the flux value of Gruber was determined from only a 5% increase of the total counting rate; the increase shown by Baker

et al. was even less than 1%. Variations of this order are easily obtained by temperature shifts, electronic instabilities or geomagnetic effects. During our flight at float the temperature inside the gondola did not change by more than 3.5 K. Even if as a consequence the gain had shifted by 20%, which is a very extreme assumption, the counting rate shown in Fig. 1 would have changed at most by 40%, which is just as large as the 2.5σ uncertainty. The geomagnetic latitude continuously decreased from 42°N to 39°N during the flight. Therefore, the flux of the Crab Nebula can at most be overestimated, but cannot be underestimated. We conclude, therefore, that the excess of γ radiation from the Crab Nebula as reported previously^{1,2} does not exist. From our upper limit one could expect that the flux accepts the value of the straight line interpolation.

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Ultraviolet photoabsorption by halocarbons 11 and 12 from electron impact measurements

DEPLETION of the stratospheric ozone layer by chlorine atoms released through solar photolysis of the chlorofluoromethanes is a matter of widespread concern¹. Predictions of the magnitude and time development of this effect depend on large scale models of atmospheric transport and photochemical kinetics which require reliable data on the primary reaction processes involved. One important input is the ultraviolet absorption spectrum of halocarbons 11 and 12, that is, CFCl_3 and CF_2Cl_2 , respectively. Published optical determinations² of the absorption spectra for these molecules between 200 and 120 nm (that is, 6.2 and 10.33 eV) have been called into question, however, by the measurements of Rowland and Molina³.

We have obtained oscillator strength distributions for a number of molecules^{4,5} from high quality electron energy-loss spectra. Previous studies have shown excellent agreement (better than $\pm 15\%$) between electron impact and optical values below 15 eV, that is, for wavelengths longer than 80 nm.

Electron energy-loss spectra for CFCl_3 and CF_2Cl_2 were obtained digitally with the NBS model AN-1 electron impact spectrometer⁵ for 100 eV electrons scattered within 20 mrad of the incident direction. Halocarbon gases were obtained commercially and used without further purification. Subsequent analysis by gas chromatography confirmed a purity greater than 99.8% with the only identifiable impurity being 0.1% CF_2ClH in CF_2Cl_2 .

Relative oscillator strength distributions for CFCl_3 and CF_2Cl_2 were derived by a procedure⁶ that corrects for the finite

angular acceptance of the apparatus. For CF_2Cl_2 (halocarbon 12) the relative distribution was then normalised at an energy loss of 12.22 eV to a value $df/dE = 0.732 \text{ eV}^{-1}$ obtained from an unpublished photoabsorption cross section of $80.3 \pm 2.4 \times 10^{-18} \text{ cm}^2$ measured by J. C. Person and coworkers at 101.4 nm. Their absolute accuracy of $\pm 3\%$ is much better than that quoted by Gilbert *et al.*⁶. Our values are within $\pm 5\%$ of the measurements of Person *et al.* between 12 and 15 eV but are nearly a factor of 1.4 higher than those of Gilbert *et al.*⁶ in this region. Between 10.4 and 11.4 eV, however, our values agree well with those of Gilbert *et al.*⁶. In fact, our disagreement with their optical data occurs mainly in the spectral region where they used a windowless absorption cell. This leads us to suspect some undetermined error in their measurements associated with the absence of windows.

For CFCl_3 (halocarbon 11) only the data of Gilbert *et al.*⁶ are available for normalisation. Since we obtain good agreement with their values between 10.4 and 11.4 eV for halocarbon 12, we have normalised our halocarbon 11 data at 10.76 eV to $df/dE = 0.906 \text{ eV}^{-1}$. This value corresponds to their molar extinction coefficient⁶ of $26,000 \text{ l mol}^{-1} \text{ cm}^{-1}$ measured at 115.2 nm. With this normalisation we found agreement with their values below 11.4 eV, but again in the windowless region our values are nearly twice as large. A more detailed analysis of the vacuum ultraviolet region for a large set of halocarbons is in preparation.

Our energy absorption results relevant to the ozone depletion problem are shown in Figs 1 and 2. A portion of our electron-impact data are compared with the optical measurements of

Fig. 1a. Comparison of oscillator strengths for halocarbon 11 (CFCl_3) in the ultraviolet absorption region: —, present electron impact results; $\times$, optical values from ref. 2; Δ , optical values from ref. 2 as quoted in ref. 3; $\bullet$, optical values from ref. 3. b. Comparison of oscillator strengths for halocarbon 12 (CF_2Cl_2) in the ultraviolet absorption region (identifications as in a).

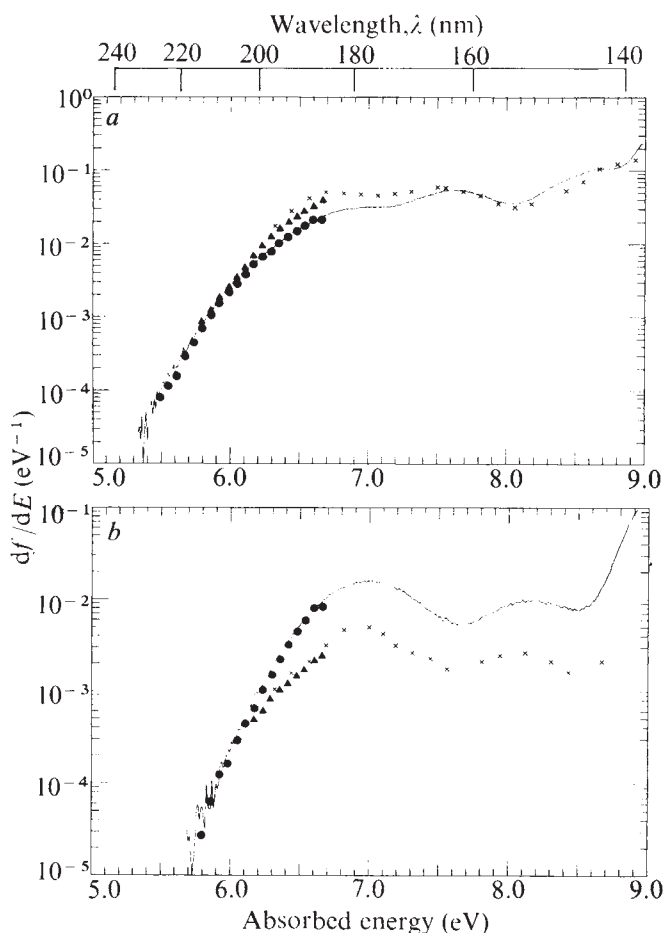


Table 1 Apparent photoabsorption cross sections for CFCl_3 and CF_2Cl_2 derived from electron energy-loss measurements

λ (nm)	Apparent photoabsorption cross section* (cm^2) CFCl_3	CF_2Cl_2
225	1.11 (−20)	—
221	2.20 (−20)	—
218	4.55 (−20)	1.97 (−21)
214	9.77 (−20)	4.50 (−21)
210	1.64 (−19)	1.08 (−20)
207	2.90 (−19)	2.63 (−20)
203	4.77 (−19)	5.16 (−20)
200	6.82 (−19)	1.02 (−19)
197	9.77 (−19)	2.10 (−19)
194	1.35 (−18)	3.44 (−19)
191	1.78 (−18)	5.65 (−19)
188	2.29 (−18)	8.42 (−19)
185	2.77 (−18)	1.15 (−18)
182	3.23 (−18)	1.49 (−18)
177	3.49 (−18)	1.76 (−18)
172	3.68 (−18)	1.39 (−18)
168	4.75 (−18)	8.91 (−19)
163	5.88 (−18)	5.98 (−19)
159	5.15 (−18)	6.87 (−19)
155	3.89 (−18)	9.46 (−19)
151	4.76 (−18)	1.07 (−18)
148	7.74 (−18)	9.45 (−19)

*The number in parentheses is the exponent of the power of ten to be multiplied by the value of the cross section tabulated

Doucet *et al.*² and the work of Rowland and Molina³. The oscillator-strength distributions we obtain for halocarbons 11 and 12 include energy absorption in the so-called "window" region of the solar radiation flux³. These data extend from the Hartley band of O_3 (maximum at 4.9 eV or 255 nm) to the Schumann–Runge continuum of O_2 (maximum at 8.5 eV or 146 nm). Our data confirm the measurements of Rowland and Molina³ for both halocarbons and disagree by as much as a factor of two for halocarbon 11 and a factor of four for halocarbon 12 with earlier measurements². Further, our results differ from those of Doucet *et al.*² over a significant portion of the absorption spectrum at higher energies for both halocarbons.

One can calculate apparent photoabsorption cross sections from the present results (Fig. 1) by multiplying by a conversion factor of $1.0975 \times 10^{-16} \text{ cm}^2 \text{ eV}$. Justification of our procedure for obtaining apparent photoabsorption cross sections from electron impact data depends on the theory of limiting oscillator strengths⁷ and is subject to the same qualifications we have discussed previously⁵. Since these data are relevant to atmospheric model calculations assessing the impact of continued halocarbon releases, the apparent photoabsorption cross sections obtained from our measurements are given in Table 1.

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extensive chemical change which takes place during this process, however, it is not clear what relationship the products have to the original structure of coal. Many workers have used oxidation procedures to degrade coal to simpler species which are more readily interpreted in terms of coal structure. Oxidation products, however, were in general studied by limited classical organic chemistry methods for separation and identification of products^{1,2}. To date the only aromatic acids definitely identified have been the carboxylic acids of benzene^{3,4}. Commonly used oxidants have been HNO₃, HNO₃-K₂Cr₂O₇, KMnO₄, O₂ and H₂O₂-O₃ in conditions for drastic degradation of aromatic rings. On the basis of results of oxidation of coal with a selective oxidant, NaOCl, Chakrabartty and Berkowitz have suggested⁵ that coal has a largely non-aromatic "tricycloalkane or polyamantane" structure. They pointed out that no evidence for aromatic compounds other than benzene derivatives was found in their oxidation product. Our experimental data do not support

Aromatic units in coal

THE organic matter in coal consists largely of a macromolecular material of complex and variable composition. When coal is pyrolysed, a large fraction is converted to char, and a number of complex aromatic compounds are formed. Because of the

Table 1 Aromatic acids found in the aqueous Na₂Cr₂O₇ oxidation of coal, and identified as methyl esters

Nucleus	Number of -COOCH ₃	Elemental composition	Precise mass (M-OCH ₃) ⁺		Relative* abundance (±15-20%)
			Observed	Deviation × 10 ³	
Benzene	2	C ₉ H ₇ O ₃	163.0400	0.6	38
	3	C ₁₁ H ₉ O ₅	221.0463	1.4	100
	4	C ₁₃ H ₁₁ O ₇	279.0516	1.2	64
	5	C ₁₅ H ₁₃ O ₉	337.0559	0.1	11
OCH ₃ -benzene†	1	C ₈ H ₇ O ₂	135.0452	0.6	17
	2	C ₁₀ H ₉ O ₄	193.0518	1.8	17
Benzophenone	1	C ₁₄ H ₉ O ₂	209.0582	-1.9	8
	2	C ₁₆ H ₁₁ O ₄	267.0677	2.0	9
Naphthalene	1	C ₁₁ H ₇ O	155.0488	-0.8	1
	2	C ₁₃ H ₉ O ₃	213.0547	-0.4	9
	3	C ₁₅ H ₁₁ O ₅	271.0610	0.6	27
	4	C ₁₇ H ₁₃ O ₇	329.0654	-0.6	8
CH ₃ -naphthalene	1	C ₁₂ H ₉ O	169.0618	-3.4	1
	2	C ₁₄ H ₁₁ O ₃	227.0711	0.3	3
	3	C ₁₆ H ₁₃ O ₅	285.0737	-2.5	5
Phenanthrene‡	2	C ₁₇ H ₁₁ O ₃	263.0699	-0.8	20
	3	C ₁₉ H ₁₃ O ₅	321.0756	-0.6	17
	4	C ₂₁ H ₁₅ O ₇	379.0813	-0.4	8
OCH ₃ -phenanthrene†	2	C ₁₈ H ₁₃ O ₄	293.0790	-2.3	8
Fluoranthene§	3	C ₂₁ H ₁₃ O ₅	345.0744	-1.8	4
	4	C ₂₃ H ₁₅ O ₇	403.0842	2.5	3
Fluorenone¶	2	C ₁₆ H ₉ O ₄	265.0517	1.8	5
OCH ₃ -fluorenone¶	1	C ₁₅ H ₉ O ₃	237.0568	1.7	1
Anthraquinone¶	2	C ₁₇ H ₉ O ₅	293.0455	0.6	8
Dibenzofuran and/or naphthofuran	1	C ₁₃ H ₇ O ₂	195.0456	1.1	18
	2	C ₁₅ H ₉ O ₄	253.0506	0.6	35
	3	C ₁₇ H ₁₁ O ₆	311.0576	2.1	28
	4	C ₁₉ H ₁₃ O ₈	369.0588	-2.0	8
Benzonaphthofuran	2	C ₁₉ H ₁₁ O ₄	303.0692	3.6	5
	3	C ₂₁ H ₁₃ O ₆	361.0738	2.7	7
Xanthone	1	C ₁₄ H ₇ O ₃	223.0397	0.3	33
	2	C ₁₆ H ₉ O ₅	281.0433	-1.6	32
Benzothiophene	2	C ₁₁ H ₇ O ₃ S	219.0122	0.6	9
	3	C ₁₃ H ₉ O ₅ S	277.0134	-3.5	1
Dibenzothiophene and/or naphthothiophene	2	C ₁₅ H ₉ O ₃ S	269.0244	-2.7	5

*Benzene tricarboxylic acid methyl ester is normalised to 100.

†Methoxy compounds may result from the reaction of phenolic compounds with diazomethane. Methoxyphenanthrene may be derived from the reaction of fluorenone with diazomethane¹⁴.

‡Although the phenanthrene ring is not affected during oxidation, anthracene is oxidised to anthraquinone⁶.

§Methyl esters of pyrene carboxylic acids show very similar mass spectra; however, the pyrene ring is extensively degraded by present procedure⁶.

¶These compounds might be oxidised further to benzene-carboxylic acids in our experimental conditions.

||It is probable that our estimates of the first two dibenzofurans are somewhat high because of contribution from fragments of xanthenes which lose both CO and OCH₃ to give the corresponding dibenzofuran ions.

Illinois bituminous coal was finely powdered, heated with 12 N HCl and washed. The same procedure was repeated three times, and the coal dried at 120 °C. The sample contained 71.33% carbon.

Table 2 N-heterocyclics found in the photochemical oxidation of coal in alkali solution, identified as methyl esters of carboxylic acids

Nucleus	Number of -COOCH ₃	Elemental composition	Precise mass (M-OCH ₃) ⁺		Relative* abundance (±15–20%)
			Observed	Deviation × 10 ³	
Pyridine	3	C ₁₀ H ₈ O ₅ N	222.0408	0.5	3.0
	4	C ₁₂ H ₁₀ O ₇ N	280.0488	3.2	3.5
Quinoline and/or isoquinoline	1	C ₁₀ H ₆ ON	156.0412	−3.7	0.5
	2	C ₁₂ H ₈ O ₃ N	214.0468	−3.5	2
Carbazole†	1	C ₁₃ H ₈ O N	194.0598	−0.7	10
	2	C ₁₅ H ₁₀ O ₃ N	252.0660	0.0	9
	3	C ₁₇ H ₁₂ O ₅ N	310.0708	−0.6	5

*Benzene tricarboxylic acid, which is the most abundant aromatic acid in this oxidation product, is normalised to 100.

†Carbazole is sensitive to oxidation with oxidising agents; however, this nucleus is stable during photochemical oxidation as shown by our own experiments with authentic carbazole and N-ethyl carbazole.

Demineralised fine powdered coal was suspended in either 10% HCl or 5% KOH aqueous solution, and oxidised with air by the radiation of a high pressure mercury lamp for 7–10 d at 40–50 °C.

this view but rather the generally accepted idea that coal is predominantly an aromatic material.

We have identified 35 aromatic carboxylic acids resulting from the oxidation of bituminous coal with aqueous Na₂Cr₂O₇ (Table 1). This oxidation procedure was chosen as it had been shown⁶ that it will oxidise substituted polycyclic aromatic compounds in good yields to their corresponding polynuclear carboxylic acids with a minimum of ring degradation. It seemed probable, therefore, that aliphatic and alicyclic linkages between aromatic units in coal would be broken, with the formation of carboxylic groups and the release of smaller aromatic units.

Illinois bituminous coal (3.6 g Peabody coal from seam No. 2) was heated at 250 °C in 120 ml 0.4 M Na₂Cr₂O₇ in a stainless steel autoclave for 36 h. The aromatic acids formed were isolated by a method similar to that of Friedman *et al.*⁶. The coal, which was almost completely insoluble in either

aqueous or organic solvents, was completely solubilised with the resulting acids weighing 53% of the original weight of coal. The acids were converted to methyl esters with diazomethane to increase their volatility for subsequent mass analysis. Individual compounds were identified by a time of flight mass spectrometer with a temperature-programmed solid inlet⁷, and verified by a precise mass determination with a high resolution mass spectrometer-computer combination. The mass spectra of methyl esters of aromatic carboxylic acids are relatively simple. The most abundant peak in practically all cases is formed by the loss of a methoxy group from the molecular ion (M-31)⁺ (refs 8–10). In addition, sizeable molecular ions and (M-COOCH₃)⁺ are observed and were used to verify the identification of each compound. Relative abundances were estimated from an integration of the base peak of each compound during the time that a sample was completely volatilised in the mass spectrometer.

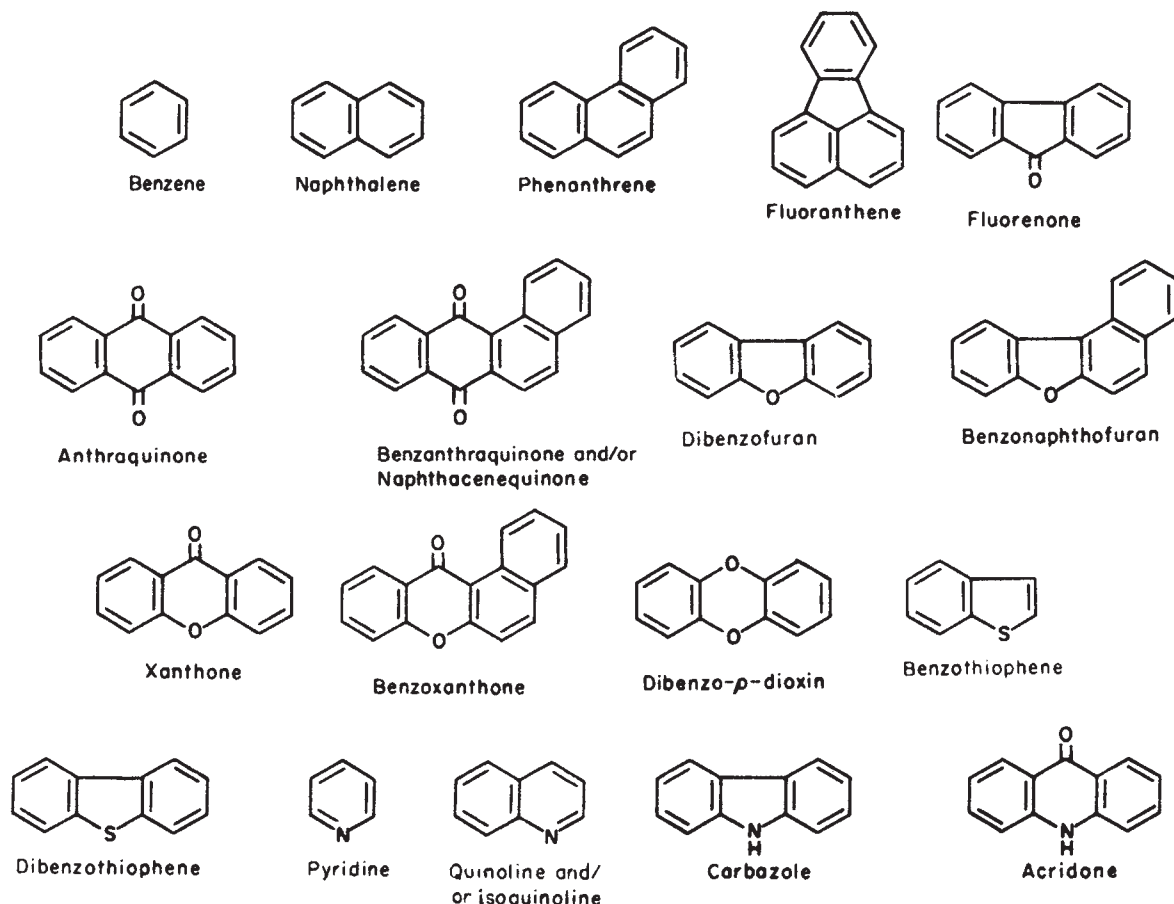
**Fig. 1** Aromatic units indigenous to coal.

Table 1 summarises the data for the methyl esters of the 35 aromatic acids which were positively identified. Another 16 oxygen-containing ions in considerable yield were observed in the mass spectra of the acid fraction but not identified.

In addition several series of interesting aromatic derivatives were observed in the chemically neutral fraction. These included dibenzo-*p*-dioxins, benzoxanthenes, benzantraquinones and acridones. These compounds exhibit an abundant M^+ as the base peak of the spectra¹¹. Amphoteric nitrogen-containing acids have not been isolated from the dichromate oxidation product because of some experimental difficulties. Seven nitrogen-containing aromatic acids produced in a photochemical oxidation were, however, identified (Table 2). Figure 1 shows the structural formulae of the aromatic units we believe to have been indigenous to the coal. Fluorenone and anthraquinone may have been produced by oxidation of fluorenes and anthracenes, respectively.

Of particular interest are the heterocyclic units of Fig. 1 with O, S, and N in ring systems. Little is known about the chemical state in coal of these important elements^{12,13} which are particularly troublesome in processes for conversion of coals to liquids and gases. Some of the aromatic units (Fig. 1) have been observed in tars produced by pyrolysis or other high temperature processes but it was not known whether these were indigenous or were produced in the process. For example benzo- and dibenzothiophene are formed when sulphur is heated with a variety of hydrocarbons. It is not likely that such compounds were formed in our low temperature aqueous oxidation experiments and thus thiophene derivatives must be indigenous to coal.

The complexity of the observed aromatic acids—with up to five carboxylic groups on benzene and up to four on polycyclic aromatics—suggests a high degree of aliphatic or alicyclic linkages between aromatic units. Such linkages are more readily oxidised than are the aromatic rings.

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First fossil records of cephalopod statoliths

THE class Cephalopoda is represented by over 10,000 fossil species and about 1,000 living species. Almost the entire fossil record so far described consists of external or internal calcareous shells of the Ammonoidea, the Nautiloidea and

some members of the Coleoidea. The subclass Coleoidea includes all but three (*Nautilus* spp.) of the living species of cephalopods, and is represented in the fossil record largely by the order Belemnitida which is important from the early Jurassic to the Eocene. Another coleoid order, the Sepioidea, including the living *Sepia* and *Spirula* have internal calcareous shells, and have left traces from the Upper Jurassic (*Voltzia*) to the present. The three remaining coleoid orders, the Teuthoidea, the Vampyromorpha and the Octopodida include all the remaining living cephalopods, comprising 29 teuthoid or squid families, one vampyromorph family and 12 octopod families.

With the exception of *Argonauta*, the 42 extant cephalopod families have no calcareous shells and such fossils as are known consist of body imprints, beaks, hooks, radulas, chitinous pens and ink sacs. Of these, beaks¹, body imprints and pens² have the most potential in relating fossils to living forms but are too few at present to allow the construction of even the most modest of phylogenetic trees.

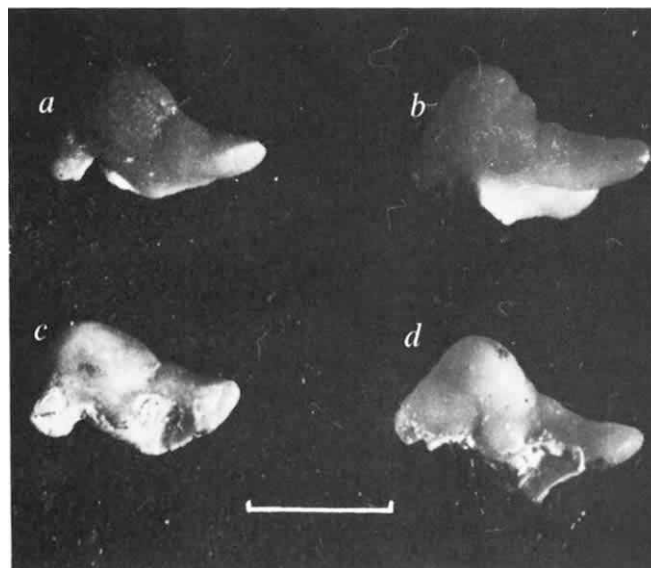


Fig. 1 *a*, The posterior and, *c*, anterior surfaces of, respectively, right and left statoliths from a Miocene deposit (Olcese Sand, California); *b*, the posterior and, *d*, anterior surfaces of respectively, right and left statoliths of a living oceanic squid, *Todarodes sagittatus*. Scale, 1 mm.

There are, however, other hard structures in cephalopods, which have so far been neglected in the study of both living and fossil forms. These are known as statoliths (= otoliths of some authors), small, paired calcareous stones with a complicated shape very characteristic of the Sepioidea and Teuthoidea, although the basic pattern varies within those groups.

Statoliths of at least one species of cephalopod are composed of aragonite, a stable form of calcium carbonate commonly found in fossils, including the fossilised otoliths of fish. It is, therefore, not surprising that cephalopod statoliths should be discovered in Cainozoic deposits. It is, however, of particular interest as it offers a possibly definitive technique for unravelling Cainozoic cephalopod phylogeny. Figure 1*a-d* compares anterior and posterior views of left and right statoliths from a Miocene teuthoid with those from an individual of the extant *Todarodes sagittatus*. Though the latter (Fig. 1*b* and *d*) are white and slightly translucent, the fossils (Fig. 1*a* and *c*) are light brown and more opaque. They will be described in more detail elsewhere.

An examination of unidentified fossil otoliths and statoliths has revealed a number of squid statoliths from North American strata. To date they have been found in the Pleistocene of California (Timms Point Silt), the Pliocene of California (Lomita Marl and Pico Formation), the Miocene of California (Olcese Sand), Florida (Chipola Formation), Jamaica (Bowden Formation), and Virginia (Yorktown Formation), and the Oligocene of Mississippi (Glendon Limestone). In one Pliocene deposit (Lomita Marl), 185 of 24,299 otoliths and statoliths recovered from approximately one ton of fossiliferous matrix represented at least three kinds of squid species. Rare fossils, very similar to modern ommastrephids *Plesioteuthis*, are known from the Upper Jurassic, suggesting that statoliths are likely to be found in strata older than the Cainozoic.

Statocysts of living cephalopods have been described by several authors³⁻⁷ but no detailed description or comparative study of the hard calcareous statolith (= otolith) has so far been published.

M. R. Clarke (unpublished) found that the statoliths of *Ommastrephes* were composed of aragonite, and in another study attempted to relate growth to the rings seen in the statoliths of *Todarodes sagittatus*. Unaltered aragonite fossils (teleost fish otoliths, mollusc shells, and so on) are abundant throughout the Cainozoic, and sometimes afford the only method of identifying faunal components^{8,9}. Indeed, fish otoliths have proved very useful in tracing the history of Recent genera, and there seems every possibility that statoliths will help clarify the relationships of Recent families, and possibly even genera, of the Cephalopoda. A study of the variation of statoliths in Recent families of cephalopods is now in progress, and it is already evident that within the limits of the general pattern the form varies considerably between and within families.

Fossil cephalopod statoliths should provide not only a means of studying phylogeny but, in addition, the relative numbers of statoliths of different families should provide an indication of the relative importance of the various families living at a particular locality and time.

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fluctuation field¹. A two-dimensional array study operated over a wide area of southern Australia has detected anomalous effects at the eastern edge of the seismically active Flinders Ranges². Various interpretations have been suggested for the Flinders anomaly² and interpretations of the accompanying seismicity³ have been given in terms of plate tectonics⁴. Independent seismic evidence⁵ indicates that there is no abrupt step in crustal and upper mantle structure from west to east across the Flinders Ranges, though there may be a gradual change. A magnetometer array in south-eastern Australia has detected anomalous effects in southern Victoria⁶, and the interpretation of a subsequent study has located the anomaly more exactly (F. E. M. L., unpublished), placing the conductor causing it under or near the Otway Ranges, another region of Australian continental seismicity. Consequently, all the major continental conductivity anomalies so far discovered in Australia are in regions of earthquake activity (Fig. 1).

The earthquakes plotted in Fig. 1 occurred between 1897 and 1972, and the distribution of observatories on the continent over this time has probably influenced the seismicity pattern shown. Several major earthquake zones are apparent, though on a world scale the seismicity of the Australian continent is low.

It is possible that the electrical conductivity anomalies and seismic zones are geographically related. Shallow conductivity anomalies could arise because fracturing has broken up the surface rocks, allowing continuity in saline ground waters to provide a conducting channel. This mechanism is, however, not entirely satisfactory for the Otway Anomaly, which may extend deeper than the seismic zone.

Beneath fracture zones, motion along fault planes presumably occurs by some type of ductile flow. Such ductile shearing of rock may increase greatly its electrical conductivity and produce a deep electrical conductivity anomaly, either by causing a change in rock fabric⁷ (for example the presence of a graphite schist has been postulated to explain a major conductive structure in North America⁸), or simply by heating. The rheological process of ductile flow beneath continental earthquake zones may involve the conversion of considerable quantities of mechanical energy to heat.

Magnetic fluctuations may in future be observed over the more northern centres of Australian seismicity (see Fig. 1) which have not yet been covered by magnetometer array studies. Because of present interest in the thermal balance of active faults⁹, heat flow data are being collated, although heat flow values would not necessarily be expected to be high: increased conductivity could result from quite a minimal increase in temperature at depth were that temperature already near the melting point, thus forming a partial melt.

If a general relationship between seismic activity and electrical conductivity anomalies is established there may be several implications for projects of earthquake prediction. The first is that deep electrical conductivity may vary both before and after an earthquake, as a consequence of associated variations in the ductile flow. (I do not mean to imply that such variations would be detected by a magnetometer array; perhaps controlled-source, electrical methods would be most suitable.) The second implication is that any observations of magnetostrictive changes in the Earth's static magnetic field may need to be corrected for non-uniform magnetic fluctuations arising from local conductivity anomalies. Such corrections may be possible if magnetostrictive observations are taken on an array basis, and full geomagnetic induction tensors¹⁰ are estimated for each observing site.

I thank J. R. Cleary, D. Denham, T. J. Fitch and L. Thomas for discussion on seismicity, and M. J. S. Johnson and P. M. Davis for discussion on earthquake prediction.

Electrical conductivity anomalies and continental seismicity in Australia

WHERE the Upper Mantle Project 'geotraverse' line in western Australia crosses seismic zones anomalous effects have been found in the vertical component of the magnetic

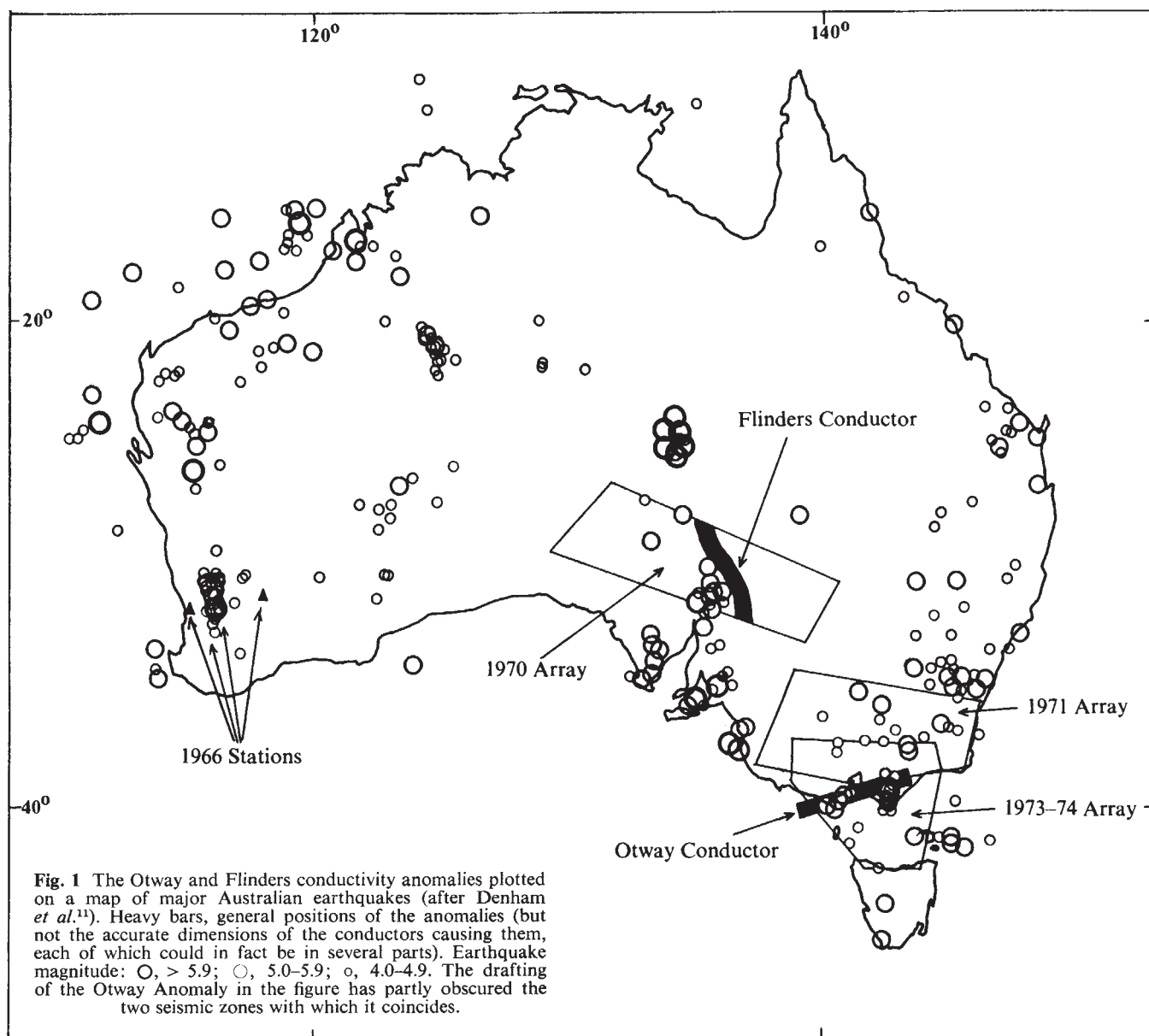


Fig. 1 The Otway and Flinders conductivity anomalies plotted on a map of major Australian earthquakes (after Denham *et al.*¹¹). Heavy bars, general positions of the conductors causing them, each of which could in fact be in several parts). Earthquake magnitude: $\bigcirc$, > 5.9 ; $\circ$, $5.0-5.9$; $\bullet$, $4.0-4.9$. The drafting of the Otway Anomaly in the figure has partly obscured the two seismic zones with which it coincides.

The Bureau of Mineral Resources provided maps of Australian earthquakes.

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Tyndall figures at grain boundaries of pure ice

LIQUID figures produced by internal melting in ice are called Tyndall figures (see refs 1-3). I report here the

formation and morphology of Tyndall figures at grain boundaries in pure ice.

I produced Tyndall figures at grain boundaries by focusing radiation from a small lamp into an area of $2 \times 2 \text{ mm}^2$ in an ice specimen which had grown from degassed, distilled and deionised water in stainless steel containers, and which had reached melting point as indicated by veins of water forming along the intersections of three grain boundaries⁴.

Two Tyndall figures with a vapour cavity were formed at a grain boundary (Fig. 1a). The vapour cavities—the black parts in Fig. 1a—are produced because of the density difference between ice and water¹. The Tyndall figures are inclined to the plane of the figure. The dashed arrows and the solid arrow (Fig. 1a) show the veins of water and the intersection of the melt grain boundary and the specimen surface, respectively. The surface of the melt boundary is not flat.

Fig. 1a shows that small perturbations are formed at the periphery of the Tyndall figures. Such perturbations were observed at the periphery of disk-shaped Tyndall figures formed in the basal plane. When the figures in the basal plane developed to a certain size, they became truncated cones and the perturbations began to be nucleated at the edge where the side face and the planar plane of the figures intersected⁵. On the other hand, the perturbation of

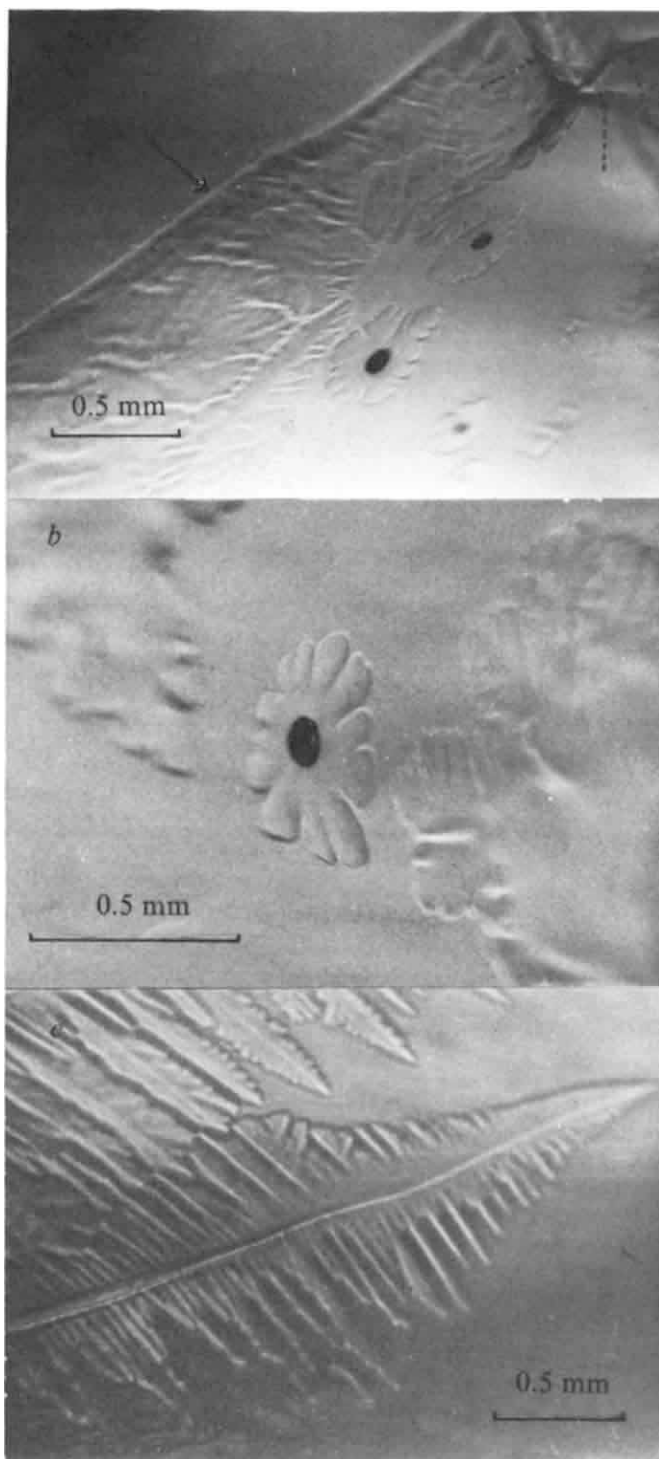


Fig. 1 *a*, Tyndall figures in a grain boundary; *b*, a 12-branched Tyndall figure; *c*, shape of a large branch of a Tyndall figure.

Tyndall figures at grain boundaries were observed even at an initial stage of their growth. Considering that the periphery of the figures at grain boundaries is always sharp because the angle where the figures meet the boundaries is 32° in the liquid phase (see ref. 4), I conclude that this kind of perturbation is nucleated at sharp edges.

During the growth of the Tyndall figures at the grain boundaries their shape changed, though some perturbations disappeared and others were nucleated. When the growth rate was large, the perturbations were sharp and their number increased. On the other hand, when the growth

rate was small, the perturbations were wide and their number decreased. The minimum number of perturbations that I observed was 12 (Fig. 1*b*).

Twelve-branched snow crystals, though rarely observed in nature, have been reported by some authors. Nakaya⁶ has examined them and concluded that they comprise two hexagonal crystals, one attached to the other. Kobayashi and Furukawa⁷ have explained their formation in terms of rotation twinning. This mechanism cannot be applied, however, to their formation because, although the orientation of adjoining grains in Fig. 1*b* could not be measured, it is clear from other observations that the morphology of Tyndall figures at grain boundaries does not depend on the orientation of the adjoining grains. Therefore, it is necessary to consider a new mechanism for the formation of 12-branched Tyndall figures.

When Tyndall figures grow large and the radiation on them becomes inhomogeneous, one of the perturbations grows preferentially and a large branch forms (Fig. 1*c*). The direction of the branch changes slightly but is usually straight. The configuration of the twigs on the branch shown in Fig. 1*c* is different from that of Tyndall figures formed in the basal plane. The angle between twigs and branches of Tyndall figures formed in the basal plane is 60° ; on the other hand, the angle between Tyndall figures at grain boundaries is 60° , 75° and 90° . The configuration of twigs on Tyndall figures at grain boundaries is similar to that observed on Tyndall figures formed in the crystallographic plane perpendicular to the basal plane (see ref. 2). This similarity may occur because the non-basal plane is less closely-packed than the basal plane.

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Photoelectrolysis of water using semiconducting TiO_2 crystals

FUJISHIMA and Honda¹ have indicated that TiO_2 can be used in a photoelectrochemical cell to decompose water with band-gap light ($h\nu \geq 3.0 \text{ eV}$). This process, termed photoelectrolysis, could have important implications for solar energy conversion and the 'hydrogen economy' if use can be made of visible light.

To understand some of the basic aspects of photoelectrolysis, we have studied the phenomena with rutile single crystals as a function of light intensity, internal cell potential and resistance, pH, and cathode material. Rutile crystal wafers (*c* axis in the wafer plane) were reduced in H_2 to produce an n-type conductivity of $0.3 \Omega^{-1} \text{ cm}^{-1}$, and were then polished, etched, and fabricated into electrodes.

TiO_2 electrodes were studied in two types of cell in which the cathodes were usually platinised platinum foil (2.2 cm^2). The first type was a homogeneous cell in which the TiO_2 and Pt electrodes were immersed in the same electrolyte. The second cell consisted of two separate compartments connected by a KCl-agar salt bridge so that the electrodes could be immersed in different electrolytes. The TiO_2 crystal was illuminated with broad band (3,000–4,000 Å) near-ultraviolet light with an intensity of

26 mW cm⁻². This irradiation was varied over four orders of magnitude with neutral density filters having absorbance (*A*) values of 1, 2 and 3.

The current-voltage characteristics of a TiO₂ crystal (area 0.76 cm², thickness 0.52 mm) were measured as a function of light intensity in the homogeneous cell using 1 N phosphate buffer (pH = 6.5). With cathodic (forward) bias, the *i*-*V* curves showed typical diode behaviour. In the third quadrant (cathodic bias and current) the results were independent of light intensity; in the second quadrant (cathodic bias and anodic current) the results depended on light intensity in the usual manner.

The *i*-*V* data for anodic (reverse) bias is shown in Fig. 1. In the dark and at low illumination levels (*A* = 3 and 2), the

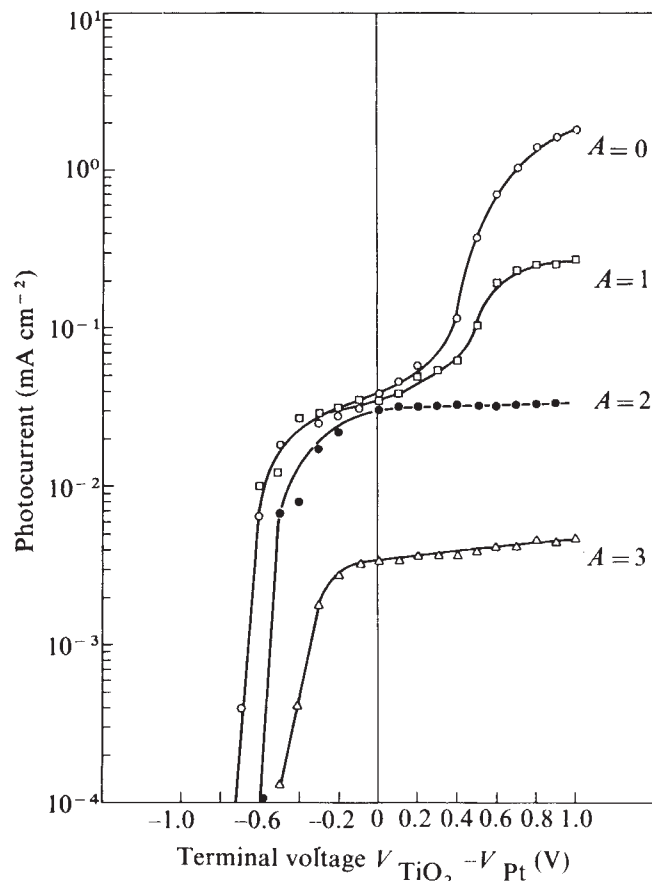


Fig. 1 Current-voltage characteristics for a TiO₂ crystal electrode in 1 N phosphate buffer (0.33 M Na₂HPO₄ + NaH₂PO₄) as a function of light intensity (homogeneous cell). The dark current is essentially zero (< 10⁻⁴ mA) with anodic bias; *A* = 0 corresponds to 26 mW cm⁻².

curves again show normal diode behaviour. The current is determined by the availability of holes in the TiO₂, and this is proportional to light intensity. At higher intensities, however, two important changes occur: (1) when the bias is between zero (short circuit) and +0.3 V the photocurrent saturates at light intensities ≥ 0.26 mW cm⁻² (*A* = 2), and (2) above a critical bias of ~ +0.3–0.4 V the photocurrent at *A* = 0 and 1 undergoes a sharp increase with bias accompanied by visible H₂ and O₂ evolution at the cathode and anode, respectively. At about +1.0 V, the photocurrent again saturates with respect to bias, but is now approximately proportional to light intensity.

The electrode potentials (against a standard calomel electrode (SCE)) for both TiO₂ and Pt are plotted in Fig. 2. As expected, the Pt potential is independent of light intensity, and above the critical bias exhibits the normal value for H₂ evolution.

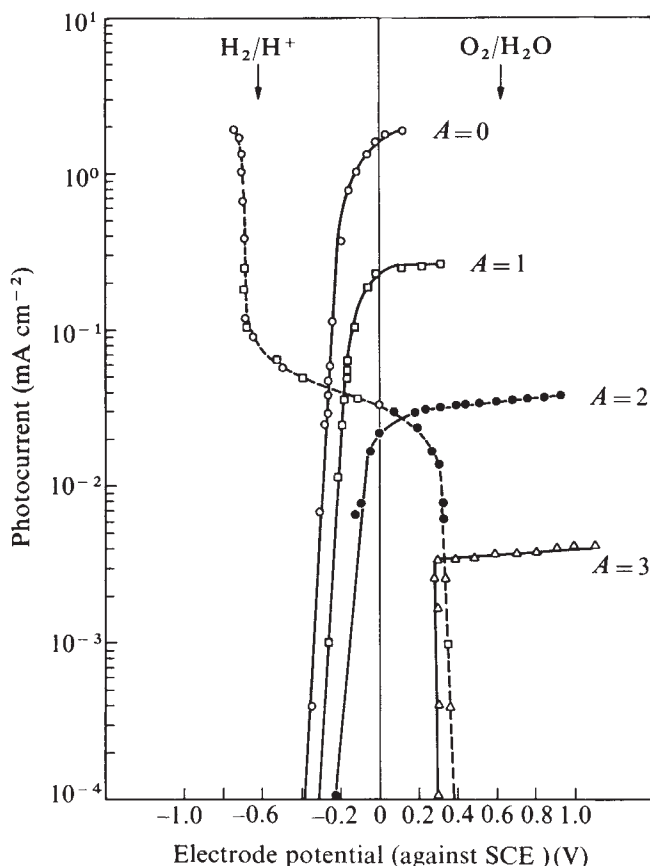


Fig. 2 TiO₂ and Pt electrode potentials as a function of light intensity in the homogeneous cell (1 N phosphate buffer). The normal H₂ and O₂ evolution potentials are indicated. ---, $V_{Pt} - V_{SCE}$; —, $V_{TiO_2} - V_{SCE}$.

The TiO₂ electrode potential depends on light intensity. At 26 mW cm⁻², the O₂ evolution potential is -0.26 V; the normal value is +0.62 V. Lower illumination intensities produce smaller negative shifts of the O₂ potential. For H₂ evolution to occur with zero bias, the potential for O₂ evolution on TiO₂ must be shifted to the negative side of the H₂ potential (a shift of -1.23 eV in a homogeneous cell).

The necessity for anodic bias to produce gaseous evolution can be understood by considering an energy balance for the absorbed photon². For band-gap photons ($h\nu \geq E_g$):

$$E_g + E_b = V_B + (\Delta G/2F) + V_H + iR + (E_c - E_f) + \eta_{Pt} + \eta_{TiO_2} \quad (1)$$

where E_g is the band gap (3.0 eV), E_b the bias, V_B the potential barrier of the TiO₂-electrolyte junction (~ 0.8 eV), $\Delta G/2F$ is the free energy per electron for H₂O decomposition (1.23 eV), V_H is the drop across the Helmholtz layer in the electrolyte (~ 0.05 eV), iR is the ohmic loss (~ 0.05 eV), $(E_c - E_f)$ is the energy difference between the conduction band and the Fermi level in TiO₂ (~ 0.2 eV), and η_{Pt} and η_{TiO_2} are the respective electrode overpotentials (~ 0.1 eV for Pt). The estimate for the potential barrier (V_B) was determined from a Schottky-Mott plot of $1/C^2$ against E_b , where C is the capacitance of the depletion layer^{3,4}. Values indicated for the other terms in equation (1) are either well known or were calculated from basic considerations.

In equation (1), the input energy requirements for gaseous evolution are 3.3–3.5 eV. All the terms on the right, except for η_{TiO_2} , account for ~ 2.4 eV. Thus, the O₂ overvoltage, η_{TiO_2} , is estimated to be ~ 0.9–1.1 eV. This results from kinetic limitations on the rate of hole injection from the TiO₂ space charge layer into the electrolyte.

Table 1 Power balance for photoelectrolysis

Cell*	Electrolyte	Internal cell resistance (Ω)	Bias (V)	Bias power (mW)	H ₂ evolution† (output chemical power) (mW)	Optical conversion efficiency‡ (%)
Homogeneous	1 N phosphate buffer	380	1.0	0.99	1.47	2.4
			0.9	0.77	1.26	2.5
			0.8	0.55	1.02	2.4
			0.7	0.36	0.77	2.1
			0.6	0.20	0.50	1.5
			0.5	0.095	0.28	1.0
			1.0	1.40	2.07	3.4
	1 N phosphate buffer	215	0.8	0.84	1.56	3.7
			0.6	0.32	0.80	2.4
			1.0	1.37	2.03	3.4
	0.1 N KOH	220	0.8	0.86	1.60	3.8
			0.6	0.38	1.06	3.2
			1.0	1.68	2.49	4.1
	0.2 N H ₂ SO ₄	65	0.8	0.94	1.75	4.1
			0.6	0.33	0.81	2.4
Differential pH	0.1 N KOH (TiO ₂)/ 0.2 N H ₂ SO ₄ (Pt)	300	0	0	1.87	9.5¶
			-0.2	-0.16§	1.16	6.7
			-0.4	-0.12§	0.46	2.9
			+0.2	0.33	2.43	10.7
			+0.4	0.74	2.73	10.1

* Optical near-ultraviolet power input is 19.7 mW (3,000–4,000 Å).

† Output chemical power is based on heat of combustion of H₂ to H₂O(l), and equals $(3.543) \times r$ where $r = \text{cm}^3 \text{H}_2 \text{h}^{-1}$.

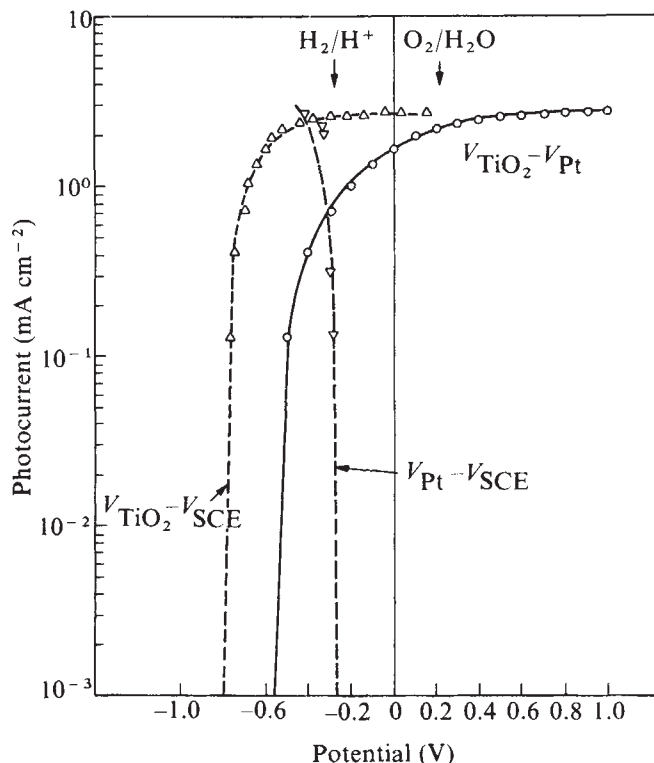
‡ Defined as (chemical power out – electrical power in)/(optical power in).

§ Electrical power withdrawn from cell.

¶ Efficiencies for the differential pH cell do not take into account the energy required to maintain the differential pH during photoelectrolysis.

One intriguing way of supplying the anodic bias is to create a protonic concentration gradient between the Pt and TiO₂ electrodes. This was done in the differential cell by placing the TiO₂ electrode in 0.1 N KOH and the Pt electrode in 0.2 N H₂SO₄. The i - V characteristic of this cell is shown in Fig. 3; at zero bias, vigorous evolution of H₂ and O₂ occurs. Gaseous evolution can be maintained even with a cathodic bias, meaning that both chemical and electrical power can be withdrawn from the cell.

Fig. 3 The i - V characteristic and electrode potentials for TiO₂ and Pt in a differential pH cell (0.1 N KOH/0.2 N H₂SO₄). The normal H₂ and O₂ evolution potentials are indicated for each compartment.



Although the rest potential of the cell is 0.78 V, no current flows in the dark under short circuit or anodic bias conditions. The electrode potentials are also shown in Fig. 3. The Pt electrode shows the normal H₂ evolution potential at all current densities. The potential for O₂ evolution on illuminated TiO₂ is shifted by ~ -1.0 V.

A power balance was made for both types of cell by measuring the input electrical and optical power, and the output chemical power (that is, the H₂ evolution rate). The results are summarised in Table 1. They show that (1) the optical conversion efficiency (OCE) is maximised at $\sim +0.8$ V for the homogeneous cell (3–4%) and at $+0.2$ V for the differential cell ($\sim 11\%$); (2) the OCE increases with decreased internal cell resistance but is unaffected by pH; and (3) the H₂ evolution rate is very sensitive to bias between $+0.4$ and 1.0 V. Additional studies of cell parameters show that the OCE increases with decreased light intensity, and that the critical bias using other metal cathodes is increased by the difference between their H₂ overvoltage and that of platinised Pt.

In summary, these studies show that the necessity of a bias for H₂ evolution arises from an O₂ overvoltage of about 1 V at the TiO₂ anode; the bias can be applied either with an external voltage or by creating a concentration gradient within the cell. The viability of photoelectrolysis with anodic bias must be examined further, especially for the cases where smaller band gap semiconductors are contemplated for improved solar absorptivity.

I note that other workers⁵ have also measured optical conversion efficiencies for TiO₂ which are comparable with the values reported here.

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Technique for the study of diffusion of large molecules in polymers based on infrared microdensitometry

THE diffusion of small molecules (in the gaseous, vapour or liquid phases) through polymeric matrices has been the subject of much study¹, and the techniques for such studies are, by and large, well established. Similar studies in the case of large molecules (broadly speaking, those forming solids at room temperature) in polymers, including polymeric self-diffusion, have been more rare²⁻⁵. Existing techniques for measurements of the diffusion of large molecules in synthetic polymers rely mainly on radioactive labelling of the diffusants. Nuclear magnetic resonance (NMR) has been used⁵, but is limited to measurements of diffusion in the melt, and also to relatively high diffusion rates⁵. Measurements involving radioactively labelled diffusants have two major shortcomings, in addition to the necessity of having to obtain the required labelled material: they fail with diffusants which are surface active with respect to the polymer², and they may involve significant interfacial resistance which the method does not reveal^{2,3}.

We have developed a simple technique for the measurement of the translational diffusion of large molecules in polymeric matrices, based on microdensitometry in the infrared. Essentially, a step function in the concentration of the diffusant is set up within the bulk matrix, and diffusion is allowed to occur at the required pressure and temperature. At some known time the diffusion is effectively terminated by quenching, and a thin slice through the composite centre is removed and scanned in the microdensitometer (Fig. 1).

To obtain a measure of the concentration of the diffusant it is necessary that it absorb at some infrared frequency unaffected by the bulk polymer, and the scanning of the thin section is, therefore, carried out at that frequency. A commercial double-beam infrared spectrometer has been adapted for the purpose, and is capable of achieving a resolution of better than 100 μm in lateral scan. Figure 2 shows the expected and observed concentration profiles for a typical run. The value of the diffusion coefficient D , is

readily evaluated from the shape of the profile⁶. It is also possible to evaluate the concentration dependence of D , where it is significant. Interface resistance, if any, is revealed by discontinuities in the broadened concentration profile⁶. Values of D in the range 10^{-2} – 10^{-10} $\text{cm}^2 \text{s}^{-1}$ may be obtained in times of the order of 1–30 d, and the estimated errors are of the order of 15%.

We have studied the diffusion of various long-chain, n -alkyl amides in low density polyethylene at about the melting temperature. The dependence on temperature and chain length is summarised in Fig. 3. The discontinuity in the slope of the Arrhenius plot as the temperature is raised is probably the result of the breakdown of the crystalline lamellae in the polythene, which at temperatures below

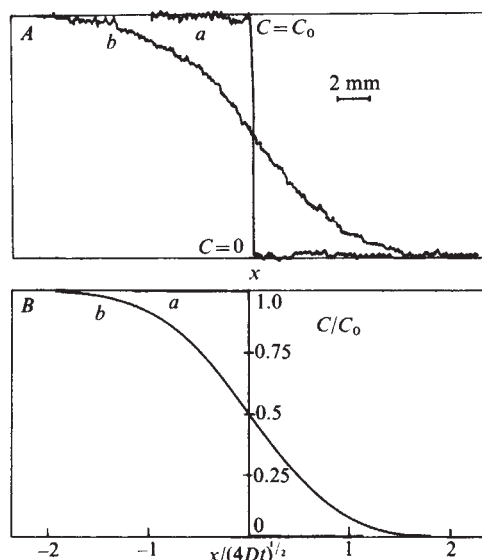


Fig. 2 Concentration profiles. *A*, observed profiles: *a*, $t \approx 0$ (initial step function; $t = 54$ s., $C_0 = 0.5\%$ stearamide in low density polythene (LDPE), weight ratio); *b*, after time t ($t = 5.22 \times 10^4$ s., $T = 130^\circ\text{C}$; C_0 as in (*a*)). Both profiles scanned at $1,660 \text{ cm}^{-1}$, an amide absorption frequency. *B*, calculated profiles⁶.

about 80°C comprise some 60% of the polymer; this leads to a decrease in the tortuosity of the path taken by the amide chains in the amorphous polymer regions, over and above the usual temperature dependence.

The diffusional behaviour of longer molecules in polymeric matrices is being explored.

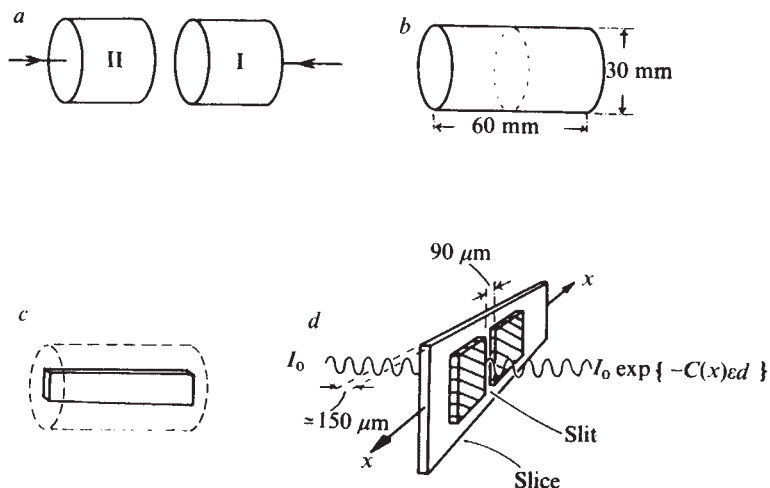


Fig. 1 Outline of experimental procedure: *a*, Specimen I—pure polymer and specimen II—polymer + diffusant, dispersed uniformly (about 0.1–1% w/w); *b*, specimen I and II joined together; *c*, slice removed from centre of composite; *d*, slice scanned in x direction for concentration profile. ϵ , Extinction coefficient of the diffusant at the chosen infrared wavelength, and the concentration $C(x)$ as x varies is recorded directly (Fig. 2) using a linear-log converter.

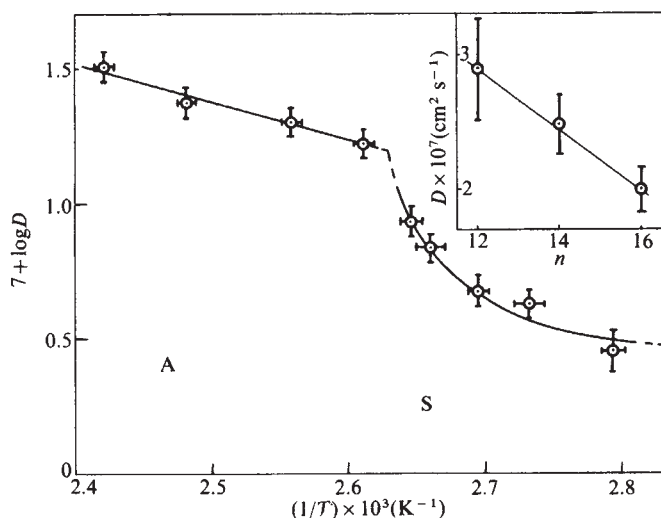


Fig. 3 Variation of the diffusion constant, D , of stearamide in LDPE as a function of temperature, T , about the melting region of the polymer: A, amorphous regime; S, semicrystalline regime. Inset, Diffusion constants, D , of $CH_3(CH_2)_nCONH_2$ in LDPE as a function of n , at $118^\circ C$. D decreases as diffusant length increases.

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Palaeotemperatures from tree rings and the D/H ratio of cellulose as a biochemical thermometer

DENDROCHRONOLOGY can provide sequences of tree rings from various parts of the world. The wood making up these rings can be accurately dated to ± 1 yr. Samples can be obtained going back a considerable time, for example wood from *Pinus aristata* (Bristle Cone Pine) trees can be obtained which goes back beyond 9,000 yr BP. If the isotopic ratios of the elements in the individual constituents in the wood contain a record of past climate, it may enable a curve of a climatic variable like temperature to be determined on a time base accurate to ± 1 yr.

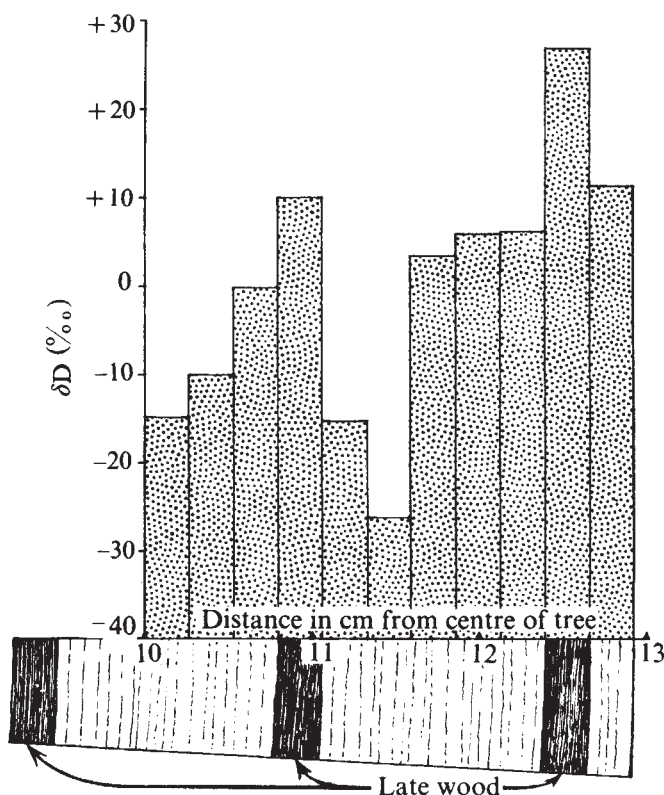
One might expect the biochemical pathways leading from CO_2 and water to the compounds that make up the wood to have isotopic effects. The question is whether the isotopic effects have significant temperature coefficients. If they do, one would have in effect a "biochemical thermometer". Here we report the existence of such a biochemical thermometer.

To study how the isotopic composition of the constituents of wood varies with temperature we have studied wood laid down during the course of one year by a *Pinus radiata* (Monterey Pine) tree growing in Hamilton, New Zealand. This particular system was chosen because at this location *P. radiata* grows throughout the year^{1,2}. Wide rings (2 cm) are laid down which enables different parts of the ring, and therefore wood laid down at different temperatures, to be sampled.

The isotopic composition of the atmospheric precipitation varies only slightly between winter and summer (about 20‰) in deuterium content³ so that any temperature effects are not masked by changing isotopic composition of the atmospheric precipitation during the year. During the year in Hamilton both the monthly mean maximum daily temperature and the monthly mean daily temperature vary through a cycle with an amplitude of $10^\circ C$. Thus the method enables even small temperature-induced isotopic effects to be detected.

In Fig. 1 we present a plot of the deuterium/hydrogen (D/H) ratio of the C-H hydrogens of cellulose across two rings laid down during the period 1919–20 by a *P. radiata* tree growing in Hamilton, New Zealand. Cellulose was prepared by the standard techniques of wood chemistry (see for example ref. 4). Before the cellulose was combusted the exchangeable hydrogens on the hydroxyl groups were brought to a standard D/H by equilibration with water of known D/H (7 d, $95^\circ C$). The results are reported in per mille deviation from standard mean ocean water⁵. The system is at least 30‰ depleted in the summer as compared with the winter and seems to record the annual temperature cycle. It should be noted that the isotopic composition of the cellulose varies in the opposite direction to the isotopic composition of the atmospheric precipitation, which is more depleted in deuterium in the winter and less depleted in the summer. Thus the effect could not be the result of the changing of the isotopic composition of the precipitation. Similarly the results could not reflect changes in the isotopic composition of cell sap since cell sap would, if anything,

Fig. 1 Variation in isotopic ratio of the C-H hydrogens in cellulose across tree rings.



become less depleted in deuterium in summer due to greater evapotranspiration associated with higher temperatures and lower humidity. It is therefore concluded that the D/H ratio of cellulose in the wood of *P. radiata* changes with temperature due to the temperature effects on one or more of the biochemical reactions leading to cellulose. Taking into account the isotopic variations of the atmospheric precipitation we obtain a temperature coefficient for the C-H hydrogens of cellulose of -5% per $^{\circ}\text{C}$.

Most probably this change reflects the air temperature since it is the biochemical pathways leading to the synthesis of the sucrose in the leaves which lead to the formation of the C-H bonds. The sucrose is translocated to the trunk where it is converted to cellulose. If it is indeed a temperature effect on the biochemical steps leading to the synthesis of sucrose, then the effect described in this communication should be found to occur generally in C-3 plants. The temperature coefficient of this biochemical thermometer could enable past temperature measurements to be measured to better than 0.1°C which should be more than adequate for studying past climate changes.

The other isotopes both in cellulose and in the other compounds in wood are being studied and will be reported elsewhere.

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Stability of Lotka-Volterra systems

NONLINEAR systems are very often studied in terms of simple mathematical models. The Lotka-Volterra equations provide such a model and have been used to study physical, chemical, ecological and social systems¹. An important question in the analysis of these equations is the stability of the equilibrium values of the interacting variables. Most work with Lotka-Volterra models has considered only neighbourhood stability against small perturbations away from equilibrium. The question of global stability when large perturbations from equilibrium are involved has only been analysed in a few special cases². We establish here that for the general Lotka-Volterra models local stability ensures global stability (asymptotic stability in the large). This result provides justification for the work that has been and is being done^{2,3} on linearised versions of the Lotka-Volterra models.

Our emphasis here will be on ecological systems: we consider a set of interacting biological species, but that does not restrict the analysis. If N_i is the biomass (or population) of species i , it changes with time t according to the generalised Lotka-Volterra equations

$$dN_i/dt = N_i \left(b_i + \sum_j a_{ij} N_j \right)$$

Here b_i are growth rates in the absence of interactions, and a_{ii} and a_{ij} ($i \neq j$) are intraspecies and interspecies interaction coefficients, respectively. We make no assumption about the

elements a_{ij} , except that they are real. Equilibrium values of the biomass, N_i^* , are defined by $dN_i/dt = 0$, or

$$b_i = - \sum_j a_{ij} N_j^*$$

We consider 'feasible' models, where all $N_i^* > 0$.

A local stability analysis is carried out by introducing small perturbations $y_i = (N_i - N_i^*)$, and obtaining a linear system $dy/dt = Ay$. Here y is the vector of y_i , and the 'community matrix' A has elements $(a_{ij} N_j^*)$. This system is stable, or equivalently the matrix A is stable, if all of the eigenvalues of A have negative real parts. The stability of A guarantees the local stability of the system about the equilibrium values N_i^* .

To examine global stability, we first introduce variables x_i through the substitution $N_i = N_i^* \exp(x_i)$. The x_i then obey the equation

$$dx_i/dt = \sum_j a_{ij} N_j^* [\exp(x_j) - 1]$$

Let x be the vector of x_i . Consider the function⁴

$$V(x) = \sum_i [\exp(x_i) - x_i - 1]$$

Observe that $V(x)$ is positive definite, continuously differentiable, and that $V(x) \rightarrow \infty$ as $|x| \rightarrow \infty$. The time derivative is

$$dV(x)/dt = \sum_{i,j} [\exp(x_i) - 1] a_{ij} N_j^* [\exp(x_j) - 1]$$

Now, if the derivative dV/dt is negative definite, the function $V(x)$ would be a Lyapunov function for the system, and would ensure global stability about the equilibria N_i^* .

The conventional way of writing quadratic forms is in terms of a symmetric matrix. So we define a symmetric matrix $B = (A + A')/2$, where the prime indicates the transposed matrix. Then we rewrite dV/dt as a quadratic form with the elements of B , b_{ij} , as

$$dV/dt = \sum_{i,j} [\exp(x_i) - 1] b_{ij} [\exp(x_j) - 1]$$

Now if B is a negative definite matrix, dV/dt is negative definite, and V is a Lyapunov function.

The theorem of Lyapunov⁵, states that a matrix A is stable (in the sense that the real parts of its eigenvalues are all negative), if, and only if, there exists a positive definite matrix C such that $(CA + A'C)$ is negative definite. We choose $C = I/2$ where I is the unit matrix. With this choice, our earlier matrices A and B are related by $B = (CA + A'C)$. Thus the theorem states that local stability (stability of our matrix A) occurs if, and only if, B is negative definite. And the negative definiteness of B makes $V(x)$ a Lyapunov function, ensuring global stability.

Thus for the general Lotka-Volterra systems, local stability implies global stability, and vice versa. This result is a happy consequence of the general form $dN_i/dt = N_i F_i(N)$ and the linearity of the F_i . This result does not apply to situations where the 'community matrix' A is critical, that is, has eigenvalues with vanishing real parts. The special cases mentioned earlier for which global stability has been studied follow from the insertion of specific assumptions about the interaction coefficients. The implications for any specific system must of course be considered within the definite limitations of the Lotka-Volterra model as a representation of reality.

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Recovery process of the sensitive plant

THERE is a long history of study of the unusual behaviour of the 'sensitive plant', *Mimosa pudica* L., stretching back to the *Enquiries into Plants*, written by Theophrastus in about 300 BC. A large literature has built up, much of which can be traced from the papers of Pickard¹, Sinyukhin and Gorchakov², and Weintraub³. In spite of this, very little work seems to have been done on the detailed kinematics of the recovery of the plant following a collapse. We report here the results of a study of the recovery of the primary petiole after seismonastic stimulation, that is, after collapse induced by a blow.

Plants used in this study were all grown from seed obtained from commercial seedhouses. The temperature was kept in the range $21 \pm 3^\circ\text{C}$ by suitable thermostatic controls. The lighting was supplied exclusively by a battery of eight 35-W fluorescent tubes mounted horizontally 24 cm above the plant pots. The sequence used was about 12 h light followed by 12 h darkness, the dark period being from 1700 to 0500. Plants were investigated at various ages in the range 1–18 months. They were grown in John Innes number 2 mixture, germinated in closed polythene containers and then transplanted into 2-inch pots.

Preliminary films of the collapse and recovery of plants taken 'end-on' to various primary petioles showed that the motions of the latter were always planar, although the motions of the secondary pinnae were more complex and sometimes gave an illusion of non-planar motion of the primary petiole. It was therefore convenient to place the plant in front of a vertical screen covered with 1-mm graph paper and orientate it so that the plane of motion of the primary petiole on which attention was to be focused was roughly parallel to the screen. A Sankyo CME 660 Super-8 cine camera was mounted so that its optical axis was roughly normal to the screen and passed close to the primary pulvinus connecting the stem of the plant to the primary petiole of interest. Preliminary experiments showed that the collapse takes a couple of seconds, whereas the recovery can take an hour or more. The camera was therefore run at its normal speed of 18 frames s^{-1} while the plant was being stimulated and collapsing, but thereafter only one frame was used every 5 s to obtain time-lapsed film of the recovery. As a check on the timing a stopwatch calibrated in 0.1-s intervals was mounted near the plant and filmed with it.

Since in most cases the primary petiole is approximately straight at all times the angle θ which it makes with the horizontal provides a quantitative measure of its geometrical condition. The graph paper on the screen was of assistance in measuring this angle. Any error in its measurement due to the direction of observation not being quite normal to the plane of motion would be small and would not affect either the general form of the θ -time curves or the periods of any oscillations contained in these.

Detailed observations were made of numerous collapses and recoveries using the plants at various ages and at a variety of times of day. The collapse process, which was always complete within 2–3 s, showed no particularly surprising features (detailed quantitative study will be reported elsewhere). The recovery process on the other hand, which usually took about an hour for completion, showed some rather unexpected features. The curve of θ against time for the recovery could take any one of three different forms which showed strong similarities to the displacement-time curves for an unforced damped harmonic oscillator. The first two forms (Fig. 1a and b) are similar to the two possible types of behaviour of a heavily damped harmonic oscillator, and accounted for 10 and 30%

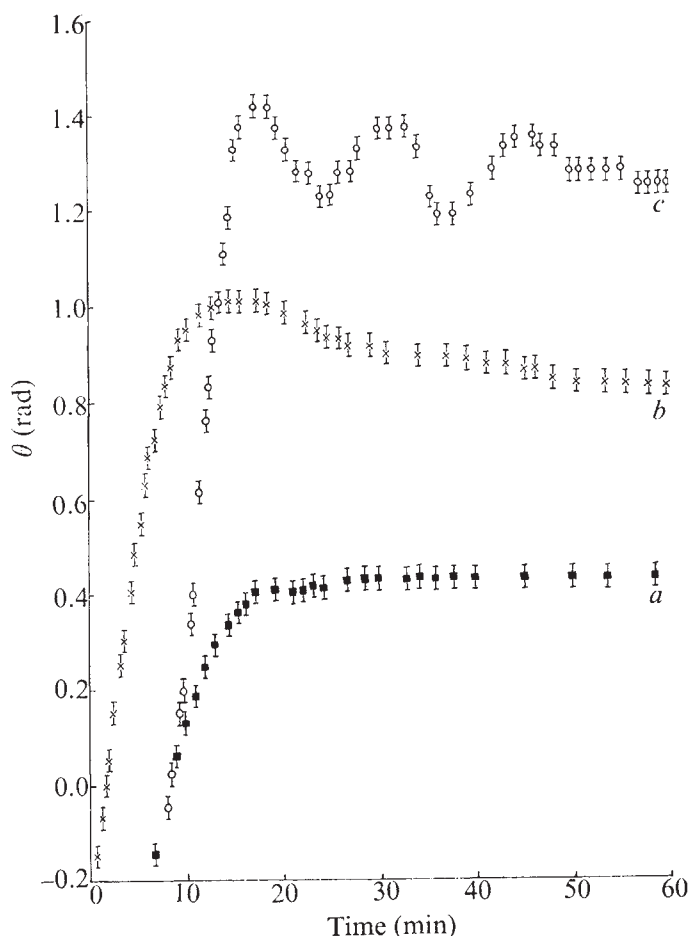


Fig. 1 Examples of the three types of recovery: a, heavily damped mode (undershooting type); b, heavily damped mode (overshooting type); c, lightly damped mode.

of the observed recoveries, respectively—referred to jointly as the heavily damped mode. The third form (Fig. 1c) is similar to the lightly damped harmonic oscillator curve. The period of oscillation was typically about 15 min. It accounted for the remaining 60% of observed recoveries. This similarity to the damped harmonic oscillator does not extend to the fine detail. In the lightly damped mode, for example, the successive displacement maxima and minima do not obey the log. dec. rule. In view of the complex internal structure of the primary pulvinus it is not surprising that the response should deviate somewhat from that of a damped harmonic oscillator: the surprise is that it should show any resemblance to it at all. In fact it seems quite likely that the system can be modelled by the usual spring-mass-dashpot system with some simple nonlinearities imposed—for example, anisotropic damping.

In addition to the main observation that a primary petiole behaves in a manner resembling a damped harmonic oscillator a number of other interesting facts emerged from our study. First, different primary petioles of the same plant can recover by way of different modes at the same time. Second, a given petiole at a given age always recovers by way of the same mode. If this mode happens to be the heavily damped one, however, its type may change. Third, on the first stimulation after a long rest period (say, at least 24 h) the final equilibrium position reached differs from that before stimulation, but immediate restimulation produces no further change in the equilibrium position. Finally, for a given petiole recovering by way of the lightly damped mode the period of oscillation is greater on second and subsequent stimulations than on the first after a long rest period. In a typical case we found that the initial period was about 15 min but that this increased by about 30%

on the second stimulation with little further change on the third.

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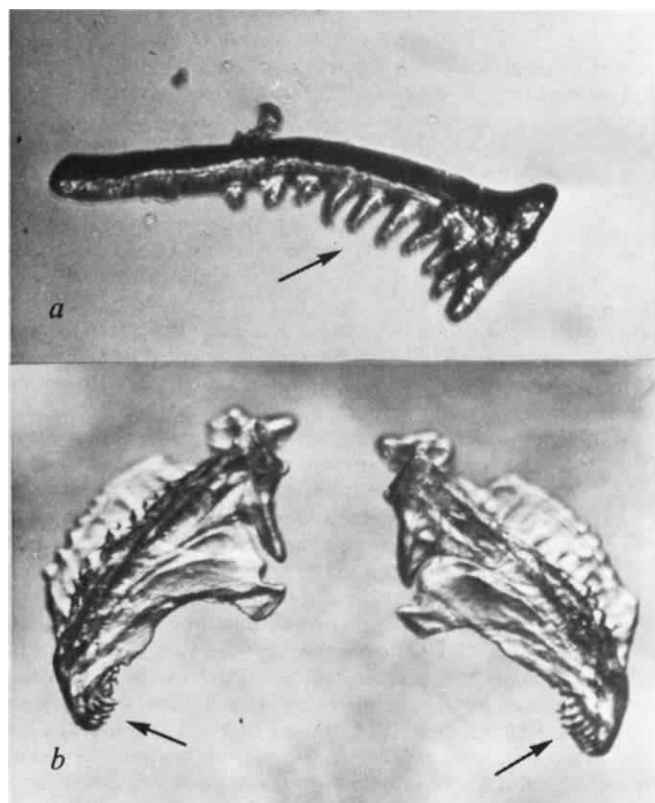
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Odontoid processes in pipefish jaws

ABSENCE of jaw teeth of any kind has been considered a familial character in pipefishes (Syngnathidae)^{1–3}, and their feeding mode has generally been described as a picking or sucking process resulting from rapid intake of water through the elongate snout^{4,5}. During systematic studies of Indo-Pacific syngnathids, we have found toothlike processes on the premaxillae and dentaries in three genera of abdominal-pouch pipefishes (Gastrophori). These structures (Fig. 1) are best developed in *Choeroichthys sculptus* (Günther) and *C. brachysoma* (Bleeker) where they are readily seen under $\times 30$ magnification in all subadults and adults. In *Syngnathoides* Bleeker (dentaries only) and a newly described genus⁶, they are inconspicuous, and best seen in cleared and alizarin-stained material.

Histological sections (from *Choeroichthys sculptus*) show no evidence of enamel, pulp cavity or basal differentiation in these processes and they appear to be odontoid projections of bone, rather than true teeth. Nevertheless, location and gross morphology suggest that they serve as functional teeth, and that they may be used in browsing or some other previously unreported mode of pipefish feeding. Data on food items are unavailable, but the habitats of these forms lend some support to this

Fig. 1 Odontoid processes of *Choeroichthys sculptus*. *a*, Right premaxilla, maximum length about 0.8 mm; *b*, mandibles, maximum length about 0.9 mm. Arrows indicate 'teeth'.



assumption. All *Choeroichthys* species and the undescribed genus are associated with coral or rocky bottoms; *Syngnathoides*, monotypic, is reported to live on weeds³. Each of these habitats would provide suitable niches for grazing pipefishes.

These odontoid processes constitute a newly recognised character of systematic importance and may prove significant in studies of pipefish phylogeny.

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Corpus allatum and ovarian growth in a polymorphic paedogenetic insect

IN parthenogenetically reproducing *Aphis craccivora* Koch, ovarian growth is initiated before birth and continues throughout larval life¹. Consequently, maintenance of the juvenile form and reproduction occur simultaneously in this insect. The corpus allatum has been shown to be active in both these processes in other insects where ovarian growth does not commence until after the larval stages are completed². To elucidate the role of the corpus allatum in the two simultaneous processes in *A. craccivora*, I have studied the gland and ovarian development during the larval stage. Both apterous and alate aphids were used as these two morphs have a different ovarian growth pattern in *A. craccivora*³.

Aphid ovaries were examined from birth to the onset of larviposition by dissection in distilled water after rapid fixation in Gilson's fluid. An index of ovarian development was found by calculating the total length of oocytes and embryos present in the body. Aphid heads were fixed in Gilson's fluid, sectioned sagittally at 6 μ m and stained with Ehrlich's haematoxylin. Corpus allatum nuclei were measured with an eyepiece micrometer and the mean nuclear size was calculated using the mean product of the long and short diameters of four nuclei in the gland³.

The mean nuclear size of the corpus allatum was positively correlated with the ovarian index in both apterae ($r = 0.91$, $P < 0.05$) and alatae ($r = 0.95$, $P < 0.05$) up to the onset of larviposition. This correlation was maintained in each morph even though their ovarian development is out of phase. Apteræ have a greater corpus allatum mean nuclear size and ovarian index than alatae during larval life until the adult moult when larviposition occurs in apterae. But the two parameters continue to increase in alatae until larviposition occurs approximately 2 d after the adult moult.

The growth of the corpus allatum during the larval stage was enormous compared with that of body length and head width. Corpus allatum volume increased elevenfold from birth to the final moult while body length and head width showed a fourfold increase. The mean length of the terminal ovarian follicle increased tenfold in the same period.

My results provide strong evidence that the corpus allatum in *A. craccivora* is simultaneously involved in ovarian growth and maintenance of the juvenile form during the larval stage. Further proof for the extra role of the corpus allatum during larval life in *A. craccivora* compared with non-paedogenetic insects is given by the measurements of relative growth of the gland and other body parts. A comparative study of several

holometabolous and hemimetabolous insects² showed little growth of the corpus allatum during the larval stage compared with the increase in body length and head width—the opposite result to that obtained with the paedogenetic *A. craccivora*. As ovarian growth and maintenance of the juvenile state are simultaneous processes in this aphid it is likely that only one corpus allatum hormone is involved in both processes.

The comparison of apterous and alate aphids correlates well with previous studies on the role of the corpus allatum in wing polymorphism⁴ and the total involvement of the gland in aphid physiology is now more apparent. The higher corpus allatum activity in apterae during the larval stage promotes faster ovarian growth and preserves the juvenile form, while in alatae, the development of wing buds and reduced ovarian growth are both coincident with a lower corpus allatum activity.

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Silent allele as genetic basis of fucosidosis

SEVERAL individuals have been recognised as having an almost complete deficiency of the lysosomal hydrolase α -L-fucosidase (EC 3.2.1.51) in their tissues and body fluids¹⁻⁷. This condition (designated fucosidosis) is associated with the accumulation of fucose-containing glycolipids and glycoproteins in various tissues^{1,5}. The clinical manifestations include coarse facial features, progressive psychomotor regression, susceptibility to respiratory infections and severe neurological signs, including generalised spasticity. Two distinct forms of fucosidosis (types 1 and 2) have been recognised⁷. Both are characterised by a virtually complete deficiency of α -fucosidase activity when assayed with artificial substrates. Physical and mental deterioration, however, proceeds far more rapidly in type 1 and these patients do not survive beyond early childhood. Type 2 patients can survive to adult life³. Additional features which distinguish the two forms of the disease are the presence, in type 2 only, of the skin lesion, angiokeratoma corporis diffusum, and an elevation in type 1 only, of the NaCl concentration in sweat⁷.

We have described a common polymorphism of α -fucosidase in the North American population⁸. Using isoelectric focusing in acrylamide gel and a specific staining technique we were able to distinguish three distinct phenotypes in neuraminidase-treated leukocyte extracts from different normal individuals (Fig. 1). Treatment with neuraminidase converted the six or more isozymes present in the original extract to one of the relatively simple patterns shown in Fig. 1, apparently by removing sialic acid residues from the more acidic isozymes and thus converting them into the less acidic forms⁹. Family studies showed that the three common phenotypes were attributable to the existence of two codominant alleles at a single, autosomal gene locus. These alleles were designated Fu^1 and Fu^2 . Thus, individuals of phenotypes Fu 1, Fu 2 and Fu 2-1 had the genotypes Fu^1Fu^1 , Fu^2Fu^2 and Fu^2Fu^1 , respectively.

We have studied the segregation of these two common alleles at the α -fucosidase gene locus in the family of two brothers affected with fucosidosis 2. The results have thrown some light on the genetic basis of the disease. The pedigree of the patients' family is shown in Fig. 2. The three phenotypes Fu 1, 2-1 and 2 were all observed in leukocytes from family members. None of the α -fucosidase isozymes was detected in leukocytes, cultured long term lymphoid lines, fibroblasts or serum from the two affected children. With this exception,

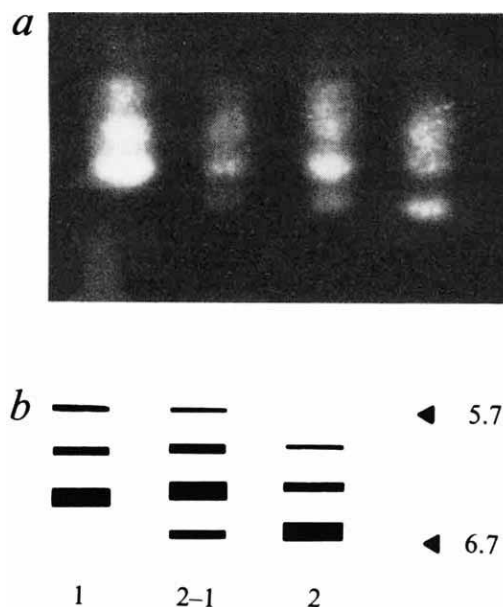


Fig. 1 Isozyme composition of the three common α -fucosidase phenotypes after isoelectric focusing of neuraminidase-treated leukocyte extracts in acrylamide gel⁸. *a*, Part of an acrylamide gel stained with 4-methylumbelliferyl- α -L-fucoside and photographed in ultraviolet light. Four samples shown have the phenotypes (from left to right) Fu 1, 2-1, 2-1 and 2. *b*, Diagrammatic representation of the three common phenotypes. Arrows show the approximate pH range within which the various isozymes focused.

isozyme patterns among family members did not differ from those seen in the general population. There exist within this family, however, two patterns of inheritance of α -fucosidase phenotypes which are clearly not consistent with the simple segregation of two codominant alleles. The first incompatibility is between the paternal grandmother (15) of the two affected children and her daughter (II4), with the mother having the Fu 2 phenotype and the daughter the Fu 1 phenotype. A photograph of the isozymes seen in leukocytes from these individuals is shown in Fig. 3. In the normal course of events the daughter would inevitably have received an Fu^2 allele from her mother and would therefore have an Fu 2 or 2-1 phenotype, depending on whether she received an Fu^1 or Fu^2 allele from her father.

If one rules out such unlikely events as fresh mutation or non-biological parenthood (particularly unlikely as it is mother and daughter who are incompatible) then the presence of a silent (or 'null') allele at the α -fucosidase locus is the only satisfactory explanation for the observed segregation of phenotypes. This allele, which we will call Fu^0 , is silent in the sense that its product (if any) is not detectable by the assay methods used in this study. Having introduced the Fu^0 allele the explanation for the observed pattern of inheritance becomes straightforward. The paternal grandmother (15) has the genotype Fu^2Fu^0 and consequently an Fu 2 phenotype. Her daughter (II4) received an Fu^0 allele from her mother and a normal Fu^1 allele from her father, resulting in the genotype Fu^1Fu^0 and an Fu 1 phenotype (Fig. 2).

The second example of apparent incompatibility between parent and child is between 12, who has an Fu 2 phenotype, and his son (III1) who is Fu 1. This situation can also be resolved by proposing that a silent allele is present. In this case the father must have the genotype Fu^2Fu^0 , resulting in an Fu 2 phenotype, whereas the son has the genotype Fu^1Fu^0 , having received a normal Fu^1 allele from his mother (11) and an Fu^0 allele from his father.

Because of the unusual inheritance of α -fucosidase phenotypes within this family we examined the segregation of phosphoglucomutase (locus 1), esterase D and red cell acid phosphatase phenotypes. All these enzymes are coded by two or

more common alleles in human populations¹⁰⁻¹². Throughout the family the inheritance of these polymorphic enzymes was completely normal and consistent with the relationships shown in Fig. 2.

If a silent allele is segregating at the α -fucosidase locus within this family it is likely that the two children affected with fucosidosis and with virtual absence of α -fucosidase activity, are in fact homozygous for this allele. If this is so then their parents must be carriers of Fu^0 and must therefore have either an Fu^1 or Fu^2 phenotype. An Fu^2 -1 phenotype is clearly impossible. As shown in Fig. 3, the two parents (II5 and II6) have the phenotypes Fu^2 and Fu^1 , respectively, presumably with the genotypes Fu^2Fu^0 and Fu^1Fu^0 .

Based on the assumptions that the three alleles Fu^1 , Fu^2 and Fu^0 are segregating within this family and that individuals marrying into the family have two normal alleles, we have been able to derive unambiguous genotypes for most of its members. In three cases, however, it was not possible to do this on the basis of phenotyping alone. Individuals I3, II8 and III3, all of whom have the Fu^1 phenotype, could be of genotypes Fu^1Fu^1 or Fu^1Fu^0 , with equal probability. In these cases the genotypes shown in the pedigree were assigned on the basis of the α -fucosidase activity of peripheral leukocytes, on the assumption that individuals carrying the silent allele should have a lower level of activity than individuals with two normal alleles. A comparison of obligatory carriers of Fu^0 (that is, I2,5,7 and II4,5,6) with normals (that is, II4,6 and II2,3,9) supports this assumption. Average α -fucosidase activity in obligatory carriers of Fu^0 was 132 ± 27 nmol per h per mg protein, whereas in normals the equivalent value was 243 ± 59 . Leukocyte activities for individual members of the family are shown in Fig. 2. The present results support previous findings which showed that a high proportion of carriers for fucosidosis (that is, heterozygotes for Fu^0) could be detected by enzyme assay methods¹³.

In each branch of the family the Fu^0 allele must have been passed down by way of the grandmother (I5 and I7) as both grandfathers are Fu^2 -1 heterozygotes and must therefore have two normal alleles. Note that whereas the two grandfathers are of Polish and German descent, the two grandmothers are both of Southern Italian parentage. Of six other families reported with fucosidosis¹⁻⁶ (four with type 1 and two with less severe type 2), three have been of Southern Italian origin (two type 1 and one type 2).

In the family described, the presence of a silent allele at the

Fig. 2 Pedigree showing segregation of common alleles Fu^1 and Fu^2 and the silent allele Fu^0 in the family of two sibs with α -fucosidase deficiency. Numbers 1, 2 and 2-1 are α -fucosidase phenotypes determined by isoelectric focusing. Leukocyte α -fucosidase activities (nmol 4-methylumbelliferone released per h per mg protein) are shown in parentheses. Two affected children are indicated by arrows. A clinical description of these two children has been presented elsewhere⁷.

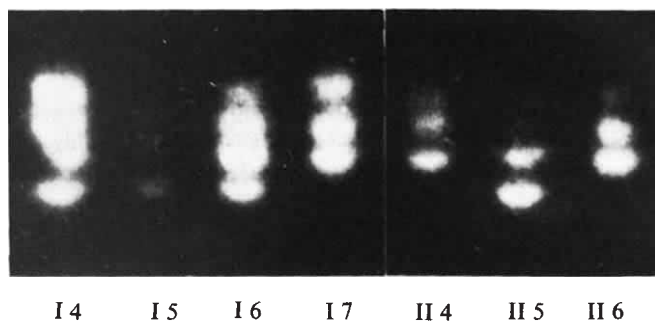
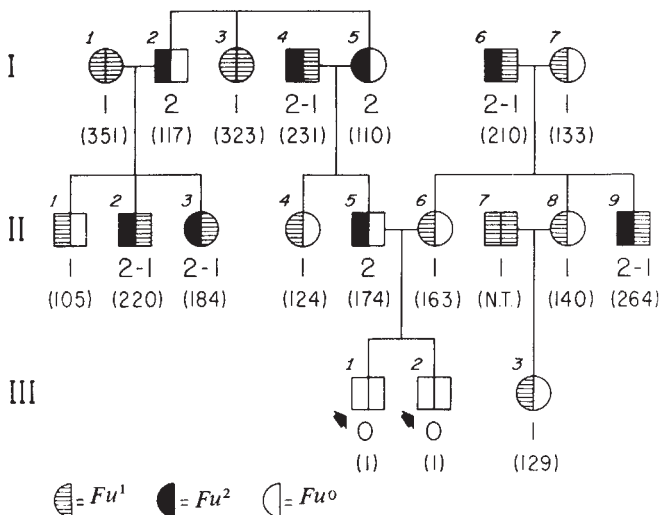


Fig. 3 Isozyme composition of α -fucosidase in leukocyte extracts from some members of the family shown in Fig. 2. Pedigree number of each individual is shown. α -Fucosidase phenotypes (from left to right) are Fu^2 -1, 2, 2-1, 1, 1, 2 and 1.

α -fucosidase locus has been deduced from apparent anomalies in the inheritance of two normal alleles at that locus. Although mutations at loci coding proteins required for activation, stabilisation or regulation of α -fucosidase could certainly account for a reduction in enzyme activity in the heterozygous state and a deficiency in homozygotes, such mutations cannot explain the qualitative abnormalities in the inheritance of the three common α -fucosidase phenotypes described here. On the other hand, a mutation within the structural locus for α -fucosidase, resulting in an inactive or silent allele at that locus, provides a satisfactory explanation for both the qualitative and quantitative aspects of α -fucosidase inheritance within this family.

Rare silent alleles have been detected during studies on the inheritance of polymorphic proteins in normal families¹⁴. Only recently, however, have family studies of this type been used to implicate structural gene mutations as a cause of human disease^{15,16}. As it has been estimated¹⁷ that 25-30% of all human enzymes have common polymorphisms detectable by electrophoresis alone, studies of this type should be widely applicable to the study of inherited enzyme deficiencies.

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Phagocytosis and cytotoxicity by a macrophage tumour and its cloned cell line

MACROPHAGES found in lymphoid and other tissues are defined by adherence, staining, morphology and rapid pinocytosis. Subtypes of macrophage-like cells have been distinguished on the basis of density and functional properties¹⁻³. Immune macrophages⁴ and normal macrophages in the presence of specific antiserum⁵⁻⁸ will lyse relevant target cells, and can phagocytose cells by an independent mechanism⁹⁻¹², but it has not been shown that the same cell type can carry out both functions.

We have described a murine reticulum cell sarcoma (J774), whose ascites form has the macrophage properties of adherence, morphology, receptors for immunoglobulin, and antibody-dependent lysis of target cells¹³. The tumour-cell pellet contains 10–50 µg lysozyme per 10⁷ cells, and as much as 200 µg ml⁻¹ of the enzyme is found in the ascitic fluid. This tumour has now been adapted to culture and cloned. Both the cell line and ascites cells exhibit phagocytosis and lysis of target cells, dependent on specific antibody, but the cell line is mainly phagocytic, and the fresh tumour is mainly cytotoxic. The result is influenced greatly by the method of assay, and the effector cell population can be rapidly switched from one mode of attack to the other. Cultured cells produce typical ascites when reintroduced into mice, but require several days *in vivo* to change from high levels of phagocytosis to cytotoxicity.

J774 ascites cells are highly cytotoxic in the presence of specific antiserum lysing 50% of target red blood cells (RBCs) in 4 h at an effector target cell ratio of 2 : 1 (Fig. 1a). Low but significant lysis is seen even at a ratio of 1 effector cell to 10 RBCs. We have shown¹³ that the small number of host peritoneal cells contained in the ascites fluid are not responsible for this killing effect. Only a small proportion of cells are phagocytosed in the same assay using tissue culture dishes. When the assay is performed in plastic tubes, the effector cells exhibit less cytotoxicity and much more phagocytosis, also dependent on specific antiserum. Wide tubes used to simulate the shallow fluid depth in culture dishes give results similar to narrow tubes. Tumour cells adhere more strongly to the dishes than tubes, and this may be a signal favouring cytotoxicity.

J774 ascites cells put into culture did not grow initially, and their cytotoxic activity gradually declined over several months. A cell line was derived after one passage back into mice, which grows partly adherent and partly floating, with a doubling time around 24 h. When subcultured separately, adherent and non-adherent cells will each generate the mixed cell population. The culture cell line also synthesises large amounts of lysozyme.

The cell line exhibits minor cytotoxicity but predominantly antibody-dependent phagocytosis (Fig. 1b). The adherent and non-adherent cells tested separately give identical results. The two culture types may represent different stages of the cell growth cycle, or a response to culture conditions, such as limited surface area, although the culture dish is not visibly crowded when floating cells appear. Most of the lysis or ingestion of RBCs occurs in the first 2 h of incubation, and radioactivity within the macrophage tumour cells is retained for more than 4 h thereafter. This culture line has been reinjected into mice many times, and the resulting ascites cells always show the strong antibody-dependent cytotoxic activity of the original tumour line (Fig. 1c).

The two effector mechanisms may be carried out by two different cell lineages coexisting in the ascites and culture populations. The growth conditions could thus influence which cell type and effector function dominated. Therefore, the culture line was cloned and tested. The cloned culture J774.1 mainly phagocytoses cells rather than lysing them (Fig. 1d), similar to the parent culture, and the ascites form of the cloned cell line mainly kills targets (Fig. 1e). Normal spleen cells, at

50-fold higher concentrations, have the cytotoxic capacity of ascites preparations, but exhibit no phagocytosis (Fig. 1f).

How rapidly can the effector cell population change its handling of antibody-antigen conjugates? Most of the killing or ingestion of RBCs by J774 occurs in the first 2 h either in the tube or dish assay using 0.5 RBCs per J774. J774 ascites cells were therefore incubated for 2 h at 37 °C in tubes which favoured phagocytosis, and then washed and tested for reaction with labelled RBCs in dishes. Most of the macrophage cells were recovered by vigorous pipetting. Preincubation alone in tubes has no effect on the subsequent ability of J774 to kill targets in dishes (90% of control killing shown by cells kept on ice during preincubation). With antiserum and 2 RBCs per J774, the subsequent killing activity was 70% of that for controls. When preincubated with antiserum and 10 RBCs per J774, during which 50% RBCs are phagocytosed with 95% of the macrophage cells containing at least one erythrocyte, the subsequent killing of newly added targets with antiserum was only 25% of that for controls. This high dose inhibition may be due to competition for macrophage surface receptor sites by antigen-antibody complexes, as well as to physiological changes resulting from massive phagocytosis.

Thus, the population of J774 effector cells can be rapidly and reversibly switched between two different mechanisms of handling target RBCs. But, the culture line still does not show the very high killing activity of the ascites preparation. To study this transition, we passaged culture cells into mice intraperitoneally and tested for antibody-dependent cytotoxicity during the next few days (Table 1). One day after *in vivo* passage, cells still resembled fresh cultured cells, whereas ascites passaged 1 d

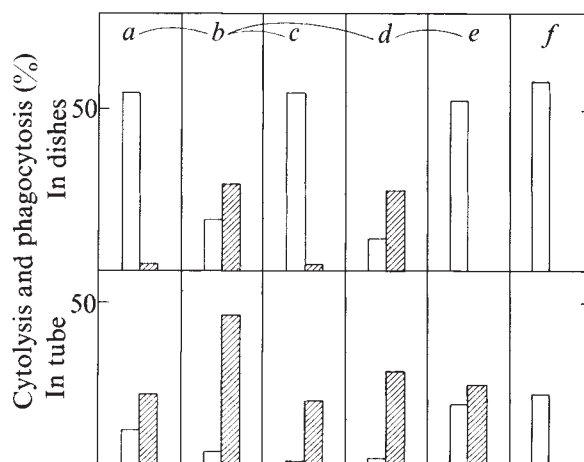


Fig. 1 Cytotoxicity (open columns) and phagocytosis (hatched columns) by J774 effector populations. Assays were performed according to Walker and Demus¹² using 2×10^5 J774 cells or 10^7 spleen cells, 10^5 ^{51}Cr -labelled sheep RBCs and 10 µl of 1:10 mouse anti-RBCs or 1:10 normal mouse serum¹³ in 1 ml volumes containing 5% heat inactivated foetal calf serum. Cultures were incubated 4 h in rocking tissue culture dishes (Falcon 3001) or stationary tubes (Falcon 2038). Plastic Petri dishes (Falcon 1008) gave less killing and phagocytosis, and large tubes (Falcon 2051), which more nearly duplicates the large area and short liquid depth of dishes, gave results identical to the small tubes. Very little effector activity was seen in assays using glass tubes. Antibody-dependent target lysis was determined by release of ^{51}Cr to the medium, and phagocytosis by water-lysis resistant radioactivity in cell pellets corrected¹³ for control values with normal serum (about 7% lysis and 14% water-resistant counts). These control values were similar to those from incubations without mouse serum or in which effector cells were replaced by 10^7 chicken RBCs. Each result is the average of several experiments using duplicates, with standard errors $\leq 7\%$ of means. *a*, *c* and *e*, Ascites cells collected from mice at 10–20 d after intraperitoneal inoculation with 0.1 ml ascites fluid (*a*) or 10^7 culture cells (*c* and *e*). *b* and *d*, J774 cells growing in Eagle's medium containing 10% foetal calf serum, after more than 100 doublings of the population *in vitro*. The cells in *d* were cloned by limit dilution in a row of 8 wells inoculated with approximately 2 cells per well, only one well developing a colony of cells, which appeared as a single focus. *f*, Normal spleen.

Table 1 Transition from phagocytosis by culture cells to lysis by ascites cells

Cells	Days <i>in vivo</i>	Effector activity			
		Dish		Tube	
		Lysis	Phagocytosis	Lysis	Phagocytosis
Culture	0	6	31	0	48
	1	11	35	0	61
	3	15	21	2	55
	10–20	54	3	11	25
Ascites	1	41	13	0	31
	10–20	55	11	6	29

Effector cell activity was assayed as in Fig. 1. J774 culture cells were used directly (day 0), or 1–20 d after transfer to mice intraperitoneally. Ascites cells were used 1 d or 10–20 d after transfer to mice intraperitoneally.

in mice showed the typical ascites response. Three days after *in vivo* passage, the culture cells showed effector levels intermediate between the culture line and long term ascites. Ascites resulting from culture cells passaged 10–20 d in mice behave as typical ascites preparations.

We believe these experiments demonstrate that a single cell lineage of macrophages can both phagocytose cells without immediate release of labelled components and directly lyse antibody-coated target cells. The evidence includes: first, the unlikelihood of two different macrophage tumours originating in the same mouse (we have seen only two reticulum cell sarcomas among more than 500 tumours during a myeloma induction programme in which J774 occurred); second, the unlikelihood of two tumours coexisting through many animal and culture passages; third, the reversible shift from lysis by ascites preparations to phagocytosis by culture cells; and finally, identical results with a cloned cell line. The assay conditions largely determine which mechanism predominates. It is not clear, however, if the same cell can carry out both functions rapidly at the same time. The effector populations studied may be composed of subgroups at different stages of differentiation. The slow transition over several days from the phagocytosis exhibited by cells grown in culture to the predominant cytotoxicity of ascites cells suggests that changes in the total population occur after one or more rounds of cell division in *in vivo* or *in vitro* conditions. It will be interesting to see if normal macrophages stimulated by growth factors to divide in culture¹⁴ can be switched from cytolysis to increased phagocytosis. An SV40-transformed macrophage cell line described by Walker and Demus¹² *in vitro* shows a high level of phagocytosis similar to cultured J774. Another macrophage-like cell line derived from a methylcholanthrene-induced 'lymphoid' neoplasm has been described by Koren, Handwerger and Wunderlich¹⁵ as being highly active in killing target cells, although a comparison of target phagocytosis was not done.

The ability to manipulate a cloned line of cells with several effector functions will facilitate further understanding of the role of macrophages in immunity.

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Resistance of *Trypanosoma cruzi* to killing by macrophages

CHAGAS' disease is a chronic debilitating illness, widespread in Latin America, caused by infection with the haemoflagellate, *Trypanosoma cruzi*. Antibodies to the organism, commonly present in both the acute and chronic forms of the disease, have generally been ineffective in protecting animals against infection^{1,2}. There is ample pathological documentation of the involvement of macrophages in Chagas' disease³⁻⁵ but unfortunately little is known about their function, or of the role of cell-mediated immunity in general. It has, however, been shown that passive transfer of immune spleen cells can protect mice against *T. cruzi* infection¹. Because macrophages serve as effector cells in protecting against intracellular parasites, we have studied the interaction of *T. cruzi* and macrophages *in vitro*. Preliminary experiments showed that at low parasite-macrophage ratios (for example 1) most cultures of normal uninduced mouse peritoneal macrophages could eliminate the infection and survive; in contrast, with higher ratios (for example 10), they were destroyed⁶. Mackaness⁷ has shown that macrophages can be 'activated' after immunisation and challenge with specific antigen to become non-specifically resistant to other infecting bacteria. We report here that nonspecifically 'activated' macrophages have heightened resistance to intracellular growth of *T. cruzi*, and suggest that its absence in normal macrophages is related to the ability of the parasite to escape from the phagolysosomes into the cytoplasm.

The Tulahuen and Ernestina strains of *T. cruzi* were supplied by Dr Franklin Neva of the National Institutes of Health, and cultured as described previously⁸. Normal macrophages were collected from the peritoneal cavity of C57BL/10.D2 mice by lavage with ice-cold Hanks' solution containing 5 IU ml⁻¹ of heparin. Macrophages which we term activated were obtained by immunising mice with living BCG (5 × 10⁵ Phipps strain, intraperitoneally) and challenging the mice 21 d later with 10⁷ BCG given intraperitoneally. The peritoneal macrophages were collected 3 d later.

The term activated macrophages has been used in the past to denote different, and not necessarily related properties, for example more rapid attachment and increased spreading on substrates, morphological changes such as highly developed membrane systems, increased lysosomal hydrolases and increased capacity to kill intracellular parasites and tumour cells. Yet, there was little to distinguish between normal and BCG-activated macrophages in terms of handling *T. cruzi* 2 h after infection; the number of organisms ingested, and their uptake into phagocytic vesicles were very similar (Fig. 1a and b and Fig. 2). While some killing of parasites was detectable as early as 2 h, by 24 h after infection at a parasite-macrophage ratio of 1 most ingested parasites were undergoing degradation intracellularly both in normal and activated macrophage populations, although at a ratio of 10 the activated macrophages seemed to be more advanced in digesting the organisms. It has been reported that living toxoplasmas can survive within mouse macrophages because they block fusion of primary lysosomes with phagocytic vesicles⁹; consequently, the Thorotrast labelling technique was used to visualise lysosomes.

Fig. 1 Fate of *T. cruzi* in normal and activated macrophages. Peritoneal macrophages from normal mice and from animals primed and challenged with BCG (activated) were cultured at 2×10^6 – 3×10^6 cells per ml in 10% foetal bovine serum on coverslips in Leighton tubes. To label phagocytic vacuoles and lysosomes, 24 h before infection or collection, macrophages were treated with a thorium dioxide (Thorotrast) suspension at a final dilution of 1:175. After washing, cultures were infected with 7-d cultures, primarily epimastigote forms, of *T. cruzi* at 1:1, 10:1 and 100:1 parasite-macrophage ratios. After 2 h, coverslips were washed three times with agitation to remove free swimming forms, and replaced with fresh medium and observed at 2, 24, 48, 72, 96 and 192 h after infection. Details for preparation of coverslips for electron microscopy have been described elsewhere⁸. *a*, *c* and *e*, Normal macrophages; *b*, *d* and *f*, activated macrophages infected at a parasite-macrophage ratio of 10. *a* and *b*, Taken 2 h after infection (primary magnification $\times 2,200$). Arrows indicate dark Thorotrast-containing vesicles (lysosomes) which have fused with phagocytic vesicles containing trypanosomes in both normal and activated macrophages. *c* and *d*, Taken after 96 h of infection (primary magnification $\times 1,320$) and indicate persistence of trypanosomes in the normal macrophages but complete elimination from the activated macrophages. Note the relative abundance of Thorotrast-containing lysosomes and vacuoles and enlarged Golgi region. Arrows indicate trypanosomes residing in the cytoplasm. *e* and *f*, Higher magnifications ($\times 3,520$) of 96-h infected macrophages. *e*, Trypanosomes in the cytoplasm of normal macrophages. Note absence of trypanosomes in Thorotrast-marked vesicles. *f*, Trypanosomal remnants, including kinetoplast within Thorotrast-containing vesicle within an activated macrophage.

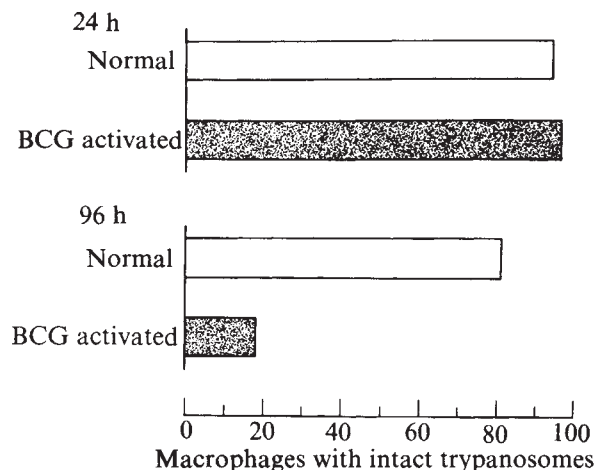
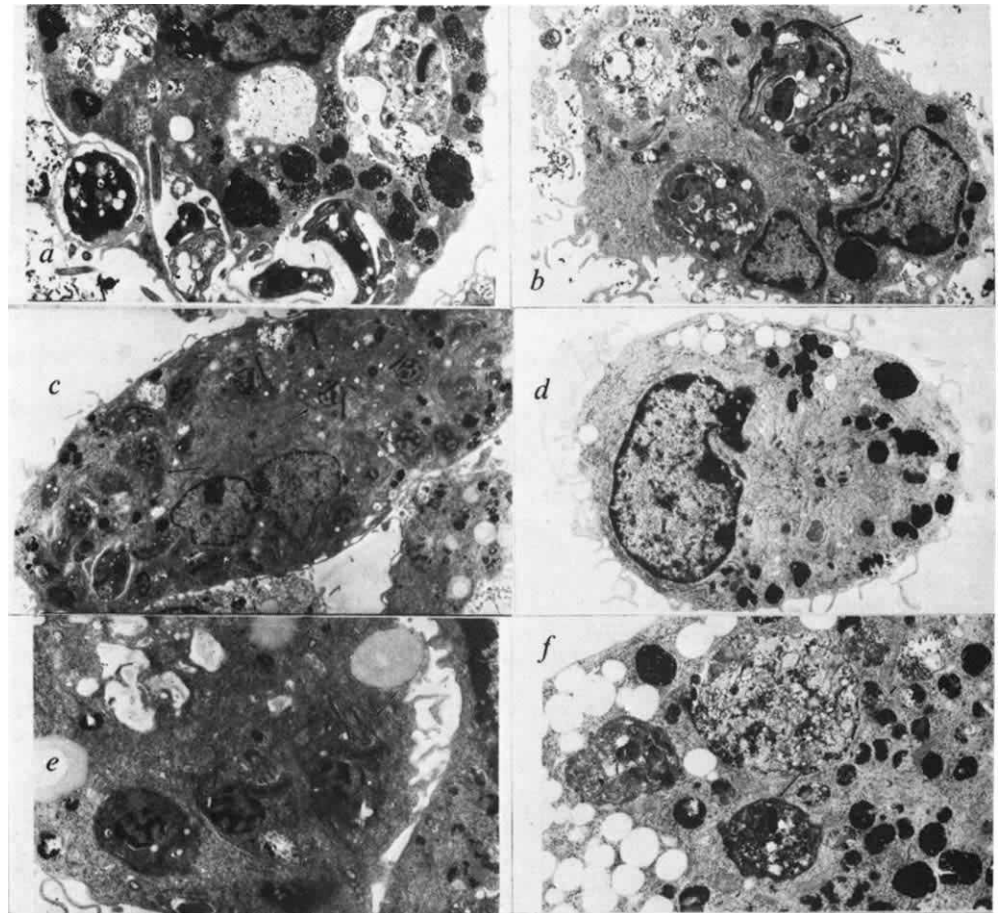


Fig. 2 Survival of intact *T. cruzi* in macrophages. Counts of intact trypanosomes were made on electron microscopic scanning of 100 consecutive macrophages from 24-h or 96-h cultures infected at a ratio of trypanosomes-macrophage of 10:1.

With *T. cruzi* virtually all parasites entered through phagocytic vacuoles which is consistent with the observation that cytochalasin B diminished the number of organisms found within macrophages⁹. Fusion of Thorotrast-labelled lysosomes and vacuoles with *T. cruzi*-containing phagocytic vesicles occurred equally well in normal macrophages and in BCG-activated macrophages (Fig. 1*a* and *b*). On the basis of observation of cells infected for 2–24 h, it would be impossible to predict which cultures were likely to be more effective in clearing the infection.

When infected cultures were examined 4–6 d after infection, however, a considerable difference was observed in *T. cruzi* growth in normal relative to activated macrophages (Fig. 1*c* and *d* and Fig. 2). Virtually all macrophages in the BCG-activated population infected at parasite-macrophage ratios of 10 had killed and degraded the intracellular parasites. The cytoplasm consisted of Thorotrast-labelled lysosomes, hyperactive Golgi and developed membranes, with occasional remnants of trypanosomes (Fig. 1*d* and *f*). In contrast, normal macrophage populations showed extensive intracellular growth of the *T. cruzi* (Fig. 1*c* and *e*). Significantly, ultrastructural examination revealed that the parasites were proliferating within the cytoplasm of the cells and were no longer found in phagocytic vesicles. Essentially no Thorotrast could be seen surrounding the parasites, and only the outer membrane of the parasite could be observed with the tilting EM stage separating them from the cell cytoplasm.

It thus seems that *T. cruzi* can survive in normal macrophages

by escaping from phagocytic vacuoles into the cytoplasm where, presumably, macrophages have no specially developed mechanisms for killing. Even though most parasites are probably killed in normal macrophages, the small number which survive seem capable of escaping and proliferating intracytoplasmically. Nonspecific activation of macrophages, as by BCG, seems to enhance the efficacy of killing in these cultures.

The distinctness of the patterns of interaction between different protozoan parasites and host cells is emphasised by these studies. Although nonspecifically activated macrophages were more effective in killing *T. cruzi*, this has not been found

to be the case with *Toxoplasma gondii* unless specific antibodies¹⁰ or supernatants of activated lymphocytes^{11,12} were present. Mauel *et al.*¹³ observed that nonspecifically activated mouse macrophages were capable of enhanced killing of *Leishmania enriettii*, which is infectious for guinea pigs, but not mice, but not of *L. tropica*, which is infectious for mice.

Our results are consistent with the finding in bacterial systems⁷ that killing seems to be very rapid, whereas degradation may take longer. The only other instance of which we are aware in which protection against macrophage killing may be associated with growth of the organism free in the cytoplasm is that of human *Mycobacterium leprae*. In studies on the growth of the organism in lymph nodes draining foot pads of mice challenged with *M. leprae*, Evans and Levy^{14,15} observed that during the logarithmic growth phase of the organism, only one membrane seemed to separate the mycobacterium from the cytoplasm; in the plateau phase, presumably brought about by the development of cellular immunity in the host, it seemed that *M. leprae* was found in vacuoles containing an additional membrane, probably the lysosomal membrane. Together, these experiments emphasise (1) the importance of lysosomes in controlling intracellular parasitism; (2) the need to design chemotherapeutic agents able to act within lysosomes; (3) the possible efficacy of nonspecific, and ultimately of specific, immunisation in enhancing destruction of phagocytosed parasites.

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Suppression of cell-mediated tumour immunity by *Corynebacterium parvum*

THE use of immunoadjuvants in tumour therapy is based largely on the concept that an increased level of specific immunity against tumour-associated antigens may be achieved by nonspecific stimulation of the immune system (for review see ref. 1). Among these adjuvants, *Corynebacterium parvum* has received much attention since it represents one of the most powerful stimulants of the reticuloendothelial system in mice², and is effective in inhibiting tumour growth in several animal tumour systems (for example, see refs 3-6). The mechanisms by which *C. parvum* interferes with tumour growth have not been established and the tumour protective effect of *C. parvum* has not been entirely consistent in all systems studied. An immunosuppressive effect of *C. parvum* has been demonstrated too. In mice, the *in vitro* lymphoproliferative

Table 1 Effect of injection of *C. parvum* on the *in vitro* secondary cytotoxic response of MSV-immune spleen cells against specific targets

Experiment no.	Source of spleen cells*	Effector cell-target cell ratio		
		50:1	25:1	12.5:1
1	MSV 30	62.8	44.4	27.1
	MSV 30	58.6	38.1	16.9
	MSV 30, CP 7	16.5	9.2	7.3
	MSV 30, CP 7	13.1	9.3	4.5
2	MSV 30	80.6	70.3	50.8
	MSV 30	72.6	63.1	43.3
	MSV 30	75.5	62.6	44.6
	MSV 30, CP 7	24.7	18.4	10.8
	MSV 30, CP 7	28.7	17.2	11.2
	MSV 30, CP 7	26.6	15.0	10.9

*Mice were tested 30 d after injection of MSV and 7 d after intra-peritoneal injection of 1.4 mg CP. A total of 20×10^6 spleen cells was cultured with 1×10^6 mitomycin C-treated RBL-5 ascites lymphoma cells for 5 d.

Surviving cells were counted by Trypan blue exclusion and the cytolytic capacity of the spleen cells against RBL-5 cells was tested in a 4-h ⁵¹Cr release assay of cytotoxicity (ref. 12) at various effector cell-target cell ratios. Results represent the mean percentage ⁵¹Cr release of quadruplicate samples of individual mice in two representative experiments. Spleen cells cultivated for 5 d in the absence of RBL-5 cells showed 5-10% cytotoxicity at the 50:1 ratio and less than 5% cytotoxicity at the two lower ratios.

responses to mitogens and to alloantigens were depressed after injection of *C. parvum*. It remains to be determined, however, whether a defect measured by these general tests of T lymphocytes may also represent an indication of depressed cell-mediated immunity against tumour cells.

A specific secondary *in vitro* cytotoxic response of immune spleen cells has been demonstrated in a murine sarcoma virus (Moloney)-(MSV)-induced tumour system⁸. The secondary response was much stronger than the primary cytotoxic response in this tumour system and in its kinetics correlated better with the status of *in vivo* immunity as determined by transplantation protection studies. We now report that injection of CP markedly inhibited the ability of spleen cells to undergo an *in vitro* secondary cytotoxic response, suggesting that treatment with CP causes a depression of specific anti-tumour cellular immunity.

Primary MSV tumours were induced in C57BL/6N mice by intramuscular injection of virus (all details of this tumour system as used in our laboratory are given in refs 9-11). Tumours usually regressed after 21 d and mice were then immune to rechallenge with MSV or cross-reacting leukaemia cells for extended periods. A secondary cytotoxic response of immune lymphocytes could be elicited either *in vivo*¹² or *in vitro*⁸. For testing the *in vitro* secondary cytotoxic response, spleen cells from mice 30 d after virus injection (designated MSV 30 spleen cells) were cultivated in the presence of mitomycin C-treated ($100 \mu\text{g ml}^{-1}$, 30 min) RBL-5 ascites lymphoma cells which have been shown to possess the relevant antigens of the MSV system¹⁰. A total of 20×10^6 spleen cells and 1×10^6 RBL-5 cells was cultivated for 5 d in 5 ml of medium RPMI 1640 (supplemented with 5% foetal bovine serum and 5×10^{-5} M 2-mercaptoethanol) in 16-ounce tissue culture flasks. Subsequently, the sensitised spleen cells and appropriate controls were tested for their ability to lyse RBL-5 cells in a 4-h ⁵¹Cr release assay (details of this assay are in ref. 9).

As Tables 1-3 show, a vigorous cytotoxic response of MSV 30 spleen cells could be demonstrated after stimulating them *in vitro* with mitomycin C-treated RBL-5 cells. There was only little activity when MSV 30 spleen cells were cultivated for 5 d in the absence of RBL-5 cells (Tables 2 and 3). The response of normal spleen cells to RBL-5 cells was also very low in our culture conditions (data now shown).

To investigate the effect of *C. parvum* (formalin-killed CP vaccine (N6314) batch 1 PX 398, Burroughs Wellcome, Research Triangle Park, North Carolina) on the *in vitro* secondary

cytotoxic response of MSV 30 spleen cells, two different protocols were used. In the first, 1.4 mg *C. parvum* was injected intraperitoneally into MSV-immune mice 23 d after virus inoculation and spleen cells were tested 7 d later for their activity in the *in vitro* assay of the secondary cytotoxic response. Representative experiments are shown in Table 1. Treatment by *C. parvum* decreased the cytotoxic response of immune spleen cells by more than 70%. This was equally observed at effector cell-target cell ratios of 50:1, 25:1, and 12.5:1.

In the other protocol, 1.4 mg *C. parvum* was injected intraperitoneally into normal C57BL/6 mice, and after 8–10 d their spleen cells were mixed with MSV 30 spleen cells, to determine their effect on the *in vitro* secondary cytotoxic response. Various numbers of spleen cells from mice injected with *C. parvum* were mixed with a fixed number of MSV 30 spleen cells. As controls, spleen cells from normal untreated mice were added. As Table 2 shows, the addition of normal spleen cells at three different doses had little or no suppressive effect on the cytotoxic response of MSV 30 spleen cells. A significant depression was seen, however, when *C. parvum* spleen cells were added to MSV 30 spleen cells.

Table 2 Effect of spleen cells from *C. parvum*-injected mice on the *in vitro* secondary cytotoxic response of spleen cells from mice immune to MSV

Source and number of cells added to 20 × 10 ⁶ MSV 30 spleen cells*	Stimulating cells	
	None	RBL-5
None	8.3†	75.8
40 × 10 ⁶ normal spleen cells	6.6	63.7
20 × 10 ⁶ normal spleen cells	7.9	68.8
10 × 10 ⁶ normal spleen cells	8.1	73.2
40 × 10 ⁶ <i>C. parvum</i> spleen cells	6.1	18.3‡
20 × 10 ⁶ <i>C. parvum</i> spleen cells	7.7	30.6‡
10 × 10 ⁶ <i>C. parvum</i> spleen cells	7.8	50.7‡

*CP spleen cells were used 8 d after intraperitoneal injection of 1.4 mg into normal C57BL/6 mice. Normal spleen cells or *C. parvum* spleen cells were cocultivated with MSV 30 spleen cells for 5 d *in vitro* in the presence or absence of mitomycin C-treated RBL-5 cells and subsequently tested for their ability to lyse RBL-5 cells in a 4 h cytotoxicity assay at a 50:1 effector cell-target ratio.

†Mean % ⁵¹Cr release of quadruplicate determinations.

‡Significant ($P < 0.05$) suppression by *C. parvum* spleen cells when compared with the effect of the same number of normal spleen cells.

To investigate the nature of the suppressor cells in *C. parvum* spleens, *C. parvum* spleen cells were added to MSV 30 spleen cells after pretreatment with various techniques. These have been described in detail previously^{11,13}, and included treatment by anti-θ serum plus complement, by an iron/magnet technique, by rayon adherence columns, and by 2,500 rad X irradiation. Controls consisted of normal spleen cells treated by the same

techniques. The results of these experiments are summarised in Table 3. In four different experiments, a marked secondary cytotoxic response was seen in cultures of MSV 30 spleen cells which ranged between 64.3 and 78.4%. Addition of an equal number of normal spleen cells, either untreated or treated by any of the techniques mentioned above had little or no inhibitory effect. In contrast, *C. parvum* spleen cells consistently suppressed the cytotoxic response of MSV 30 spleen cells. Their effect was eliminated by pretreatment with the adherence columns or with the iron/magnet technique. The suppressive capacity of *C. parvum* spleen cells was not removed by pretreatment with anti-θ serum plus complement or by irradiation with 2,500 rad.

These results indicate that the cells in *C. parvum* spleens which inhibit the secondary cytotoxic response of immune spleen cells to tumour-associated antigens are radioresistant non-T cells. A role of B cells has not been definitively ruled out. The iron/magnet technique and the adherence columns as used in our laboratory, however, although effective in removing macrophages, do not selectively deplete B lymphocytes (this was shown both by stimulation with the B cell mitogen LPS and by determination of cells with detectable surface immunoglobulin). Therefore, our data suggest that the suppressor cells in *C. parvum* spleens are macrophages. This is in accord with the results of studies of the suppression of mitogen responses of normal spleen cells by *C. parvum* spleen cells⁶, in which the suppressor cells within *C. parvum* also appeared to be macrophages. In other studies, activated macrophages have been demonstrated after injection of *C. parvum* which inhibited the *in vitro* proliferation of tumour cells^{6,14}. Subsequently, we have presented evidence that in spleens of *C. parvum*-injected mice the same cells may suppress mitogen reactivity and inhibit tumour cell proliferation *in vitro* (H.K. *et al.*, unpublished). It is conceivable that activated macrophages have the same dual effect *in vivo* after injection of *C. parvum*. There may be a balance between immunosuppression and the inhibition of tumour cell growth, and it is possible that treatment schedules with the greatest activation could result predominantly in immunosuppression and consequent facilitation of tumour growth. Indeed, in one experimental tumour system, strong tumour protection occurred in conditions of minimal systemic activation, whereas there was little protection after systemic administration of *C. parvum*⁶. This lack of protection may be caused by similar suppressor cells as described here. Furthermore, it has been observed that tumour protection by injection of X-irradiated tumour cells has been diminished in mice pretreated with *C. parvum*⁵.

In conclusion, our data show the depression of anti-tumour cellular immunity by treatment with *C. parvum*. These data are not necessarily at variance with reports in which a tumour-protective effect of *C. parvum* has been shown. *C. parvum* may

Table 3 Effect of various treatments on the suppressive effect of *C. parvum* spleen cells on the *in vitro* secondary cytotoxic response of MSV-immune spleen cells

Experi- ment no.	Type of treatment*	Type of spleen cells cultured									
		MSV 30 spleen cells alone†		MSV 30 spleen cells plus‡§ untreated <i>C.</i> <i>parvum</i> spleen cells		MSV 30 spleen cells plus treated <i>C.</i> <i>parvum</i> spleen cells		MSV 30 spleen cells plus untreated nor- mal spleen cells		MSV 30 spleen cells plus treated normal spleen cells	
		+RBL-5	—RBL-5	+RBL-5	—RBL-5	+RBL-5	—RBL-5	+RBL-5	—RBL-5	+RBL-5	—RBL-5
1	Anti-θ serum plus complement	64.3¶	6.7	27.3	6.3	25.1	7.4	56.7	8.3	54.8	6.7
2	Iron/magnet	71.3	4.9	32.8	6.6	66.1	3.9	64.6	7.2	66.7	7.3
3	Rayon adherence columns	78.4	9.2	38.6	6.9	78.3	7.2	73.5	6.1	74.6	5.6
4	2,500 rad X irradiation	70.6	5.4	31.8	5.7	30.6	6.1	72.3	7.1	70.4	8.2

*Details of these treatments are contained in refs 14 and 17.

†MSV-immune spleen cells were obtained 30 d after MSV injection.

‡*C. parvum* spleen cells were obtained 8 d after injection of *C. parvum* into normal C57BL/6 mice.

§20 × 10⁶ MSV 30 and 20 × 10⁶ CP spleen cells were cultured for 5 d in the presence of mitomycin C-treated RBL-5 cells and subsequently tested for their ability to lyse RBL-5 cells in a 4-h ⁵¹Cr release assay of cytotoxicity. At least three different effector cell-target ratios were tested in each experiment. Shown here are the data at a 50:1 ratio.

¶Mean % ⁵¹Cr release of quadruplicate samples.

turn out to be a useful therapeutic agent in the treatment of cancer. Our data suggest, however, that there may be quite a delicate balance between the beneficial anti-tumour effects of *C. parvum* and its immuno suppressive effects. Considerable experimentation seems to be necessary to determine the various factors contributing to this complex situation. Therefore, caution should be exercised in using *C. parvum* for immuno-therapy of human cancer.

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Role of amino acids in osmoregulation of non-halophilic bacteria

MOST microorganisms show optimal growth at low osmotic pressures, but even so are often able to grow, though more slowly, in the presence of concentrations of environmental solutes sufficiently high to reduce the water activity to values as low as 0.86 (Table 1). The ability of osmophilic yeasts to grow at low water activities depends on the accumulation of high concentrations of polyhydric alcohols inside the cell¹, and similarly, halophilic bacteria accumulate potassium ions². Amino acids, especially proline, have been shown to stimulate growth³ and respiration⁴ of some bacteria at low water activities, and to accumulate in response to increased environmental sodium chloride⁵ and, more particularly, to osmotic dehydration (my unpublished results). I now report that growth of non-halophilic bacteria at low water activities seems to depend on the ability of the cell to balance the environmental osmotic pressure by intracellular accumulation of amino acids, and on the types of amino acid which accumulate.

Non-halophilic bacteria differ greatly in their tolerance to sodium chloride in the growth medium (Table 1, first and second columns). There are two possible explanations: (1) the cells are permeable to the solute and differences in tolerance reflect the intrinsic solute resistance of intracellular enzymes; (2) the cells are impermeable to the solute and must therefore compensate internally for the increased environmental osmotic pressure. To test the first possibility, the salt sensitivities of the enzymes, aldolase, isocitrate dehydrogenase and phosphohexose isomerase, were assayed in whole cell homogenates of bacteria with widely different salt tolerances (Table 2). Although differences were evident between species, these were not sufficient to explain the differences in halotolerance and, further, concentrations of sodium chloride which would totally inhibit all these enzymes *in vitro* still permitted growth of the intact cells of the three species tested. Complete permeation of solute into the cell therefore does not occur and a concentration

difference is maintained across the cytoplasmic membrane. Such a concentration difference between the inside and the outside of the cell would cause osmotically induced dehydration unless accumulation of some other osmotically active molecule occurred within the cell. An osmotically induced accumulation of proline in *Bacillus subtilis* had already been demonstrated (my unpublished results), and therefore analyses were made of the free amino acids within the cells of other species of bacteria which had been grown in the presence of high concentrations of sodium chloride. A concentration was chosen for each species which caused a 50% reduction in growth rate.

In each of the species tested, salt induced a characteristic increase in the levels of particular pool amino acids (Table 1, third and fourth columns). To balance 1 M NaCl outside the cell requires an internal concentration of approximately 1 M ionic, or 1.8 M non-ionic solute. In some instances the increase in intracellular amino acid concentration appeared insufficient to balance the external osmotic pressure (Table 1, fourth column). The probable explanation is, first, that bacteria are normally hypertonic with respect to the environment. (In fact the more osmotolerant a microorganism is, the greater is its hypertonicity, and more specifically its internal K⁺ concentration, when growing at high water activity⁶.) Consequently, a part of the added solute pressure need not be balanced by additional intracellular solute. Second, because glutamate is charged at neutral pH values, it is accompanied by a cation (usually K⁺), which also contributes to the osmotic balance. Third, and particularly for *Staphylococcus aureus*, Na⁺ may be imperfectly excluded and therefore contribute to the balance also.

That the changes in amino acid level are osmotically induced can be judged by the fact that the same qualitative changes are induced by sucrose in place of salt in the environment, but the increase of intracellular amino acid per mol of sucrose is roughly 45-50% of the increase per mol of sodium chloride.

What can induce these changes? They occur in a rich medium, when the cells transport amino acids from the medium, or in a minimal medium when they synthesise them *de novo*. Those organisms which accumulate glutamic acid in response to osmotic stress are mainly Gram-negative species which, under normal conditions, have low total amino acid pools composed almost entirely of glutamate and also low K⁺ levels. At the other extreme, those organisms which accumulate proline are Gram positive and have a large free amino acid pool under non-stressed conditions (of which, again, a large proportion is glutamate) and also a high internal K⁺ level. In fact, such Gram-positive organisms growing in the absence of osmotic stress have pools similar to those of Gram-negative organisms grown in the presence of, say, sodium chloride. It thus seems that, during osmotic stress, the fundamental reaction is an increase in pool glutamate. Among Gram-negative organisms there is scope for this increase, but among the Gram-positive organisms glutamate may already be at a limiting concentration. Accumulation of glutamic acid requires the cell to accumulate an accompanying cation in order to retain electrical neutrality. In most cases this is K⁺. The disadvantages of K⁺ accumulation to most species are the same as those of allowing NaCl to enter the cell—the inhibition of enzymatic processes. Gram-positive organisms have therefore evolved mechanisms to convert glutamate through two closely related pathways of metabolism to form the other osmoregulatory solutes, proline or γ -aminobutyric acid (GABA). Significantly, neither of these is highly charged at neutral pH value, and so will not require the presence of a neutralising cation.

Accumulation of K⁺ may also be involved in osmoregulation. The first effect of an osmotic stress is to remove water from the cell and thereby increase the intracellular K⁺ concentration. The enzyme involved in glutamate synthesis

Table 1 Relationship of the major osmoregulatory amino acids to osmotolerance of microorganisms

Organism	Minimum water activity* for growth in NaCl	Equivalent [NaCl] % w/v	Amino acid(s) showing major increase in free pool during growth in NaCl†	Increase in amino acid pool level per mol of environmental NaCl (mmol)‡	
<i>Pseudomonas aeruginosa</i>	0.970	5.1	Glutamic acid	900	
<i>Vibrio parahaemolyticus</i>	0.950	8.5	Glutamic acid	900	
<i>Escherichia coli</i>	0.950	8.5	Glutamic acid	150	
			GABA		300
			Proline		630
<i>Salmonella oranienburg</i>	0.950	8.5	Glutamic acid	520	
			Proline		500
<i>Clostridium sporogenes</i>	0.945	9.3	Glutamic acid	280	
			GABA		500
			Proline		420
<i>Lactobacillus plantarum</i>	0.945	9.3	Glutamic acid	420	
			Proline		830
<i>Bacillus megaterium</i>	0.945	9.3	Proline		1,330
<i>Serratia marcescens</i>	0.943	9.5	Glutamic acid	700	
			Proline		400
<i>Klebsiella aerogenes</i>	0.940	10.0	Glutamic acid	750	
			Proline		450
<i>Streptococcus faecalis</i>	0.940	10.0	GABA		750
			Proline		750
<i>Micrococcus lysodeikticus</i>	0.930	11.4	Proline		1,550
<i>Sarcina lutea</i>	0.920	13.0	Proline		1,570
<i>Bacillus cereus</i>	0.920	13.0	Proline		1,670
<i>Bacillus subtilis</i>	0.900	15.7	Proline		1,650
<i>Staphylococcus aureus</i>	0.860	20.6	Proline		1,050

* Microorganisms were grown with aeration at 37 °C in potato extract–yeast extract–glucose broth (Difco) with the following exceptions: *Vibrio parahaemolyticus* was grown in heart infusion broth (Difco); *Lactobacillus plantarum* in MRS broth (Oxoid); *Clostridium sporogenes* in reinforced clostridial medium (Oxoid) without aeration. Sodium chloride at various concentrations, as a solid, was added to exponentially growing cultures of the organisms and growth rate was determined by following absorbance at 580 nm. Minimum water activity for growth was then determined by extrapolating a graph of growth rate against sodium chloride concentration to zero growth rate. The water activity of this NaCl-containing medium was determined on a Sina equilibrium hygroscope.

† Microorganisms were grown at 37 °C in media containing sufficient sodium chloride to reduce the growth rate by 50%. At a concentration of 2×10^8 cells per ml cultures were collected by centrifugation and washed twice in 0.1 M phosphate buffer, pH 7.0, containing sodium chloride at a concentration the same as that in the growth medium. The cells were finally resuspended at a density of approximately 10^{10} per ml in ice-cold 5% (w/v) trichloroacetic acid. Amino acid analyses were carried out using a Technicon automatic amino acid analyser. For each species controls were grown to the same cell concentration in the absence of sodium chloride, then treated as above.

‡ Intracellular concentrations of amino acids are based on phosphate-impermeable volumes for each species.

in most bacteria in cultures not limited by ammonia is glutamate dehydrogenase, which catalyses the oxidative deamination of glutamate and (or) the reductive amination of α -ketoglutarate. Table 3 shows that this enzyme is activated by 500 mM K^+ up to tenfold in the direction of glutamate, but not at all in the reverse reaction in the several species tested. There is thus a cyclic build up of glutamate resulting from the osmotically effected increase in K^+ concentration leading to glutamate synthesis, which then requires inflow of cations to balance the charge, which further activate the enzyme. In organisms that accumulate glutamate, a concomitant increase in intracellular K^+ concentration is observed, and this is of the same magnitude as the increase in glutamate concentration. For example, in *Klebsiella aerogenes* grown in the absence of osmotic stress the intracellular K^+ concentration is 125 mM. During

growth in the presence of 1 M NaCl this increases to 625 mM, an increase of 500 mM. The extra glutamate accumulated under these conditions is 750 mM. Similar changes can be observed in other organisms.

In some bacteria, notably some *Bacillus* spp., glutamate synthesis occurs partly (or completely in the case of *Bacillus megaterium**) by a separate route, through glutamine synthetase (GS) and glutamine: α -ketoglutarate amidotransferase (GOGAT). The GS–GOGAT system is not activated by ions (Table 4), nor does the total enzyme level increase substantially during growth at low water activity and therefore other mechanisms must be involved. Ions may, for example, influence the enzymes of proline synthesis or inhibit feedback inhibition which normally limits the free proline pool level: and in some microorganisms the rate of transport of amino acids into the cell can be con-

Table 2 Inhibition of various microbial functions by sodium chloride

Organism	Growth*	Sodium chloride concentration (% w/v) causing 50% inhibition of:		
		Phosphohexose isomerase†	Isocitrate dehydrogenase‡	Aldolase§
<i>Escherichia coli</i>	4.4	3.75	3.5	3.75
<i>Bacillus subtilis</i>	8.1	5	5.25	4.75
<i>Staphylococcus aureus</i>	10.9	6	7	7

* Determined as in Table 1.

All enzyme assays were performed on whole cell lysates of the organisms prepared by ultrasonication of suspensions of 10^{10} cells per ml 0.1 M phosphate buffer, pH 7.0.

† Assayed according to the method of Bodansky and Calitri¹¹.

‡ Assayed according to the method of Siebert *et al.*¹².

§ Assayed according to the method of Sibley and Lehninger¹³.

Table 3 Effect of K⁺ ions on glutamate dehydrogenase activity

Organism	Substrate*	Activity† of enzyme in		
		0	250	500 mM K ⁺
<i>Escherichia coli</i>	NADPH	320	1,910	3,060
	NADP	80	75	70
<i>Klebsiella aerogenes</i>	NADPH	480	1,500	2,400
	NADP	65	65	60
<i>Bacillus subtilis</i>	NADPH	650	1,830	3,170
	NADP	25	20	20
<i>Staphylococcus aureus</i>	NADPH	400	1,520	2,810
	NADP	30	30	30

*NADPH oxidation was used as a measure of the forward reaction, NADP reduction as a measure of the reverse reaction. In the latter case 5mM glutamate was added in place of α -ketoglutarate in the standard assay procedure.

†Assayed according to the method of Meers *et al.*¹⁴.

Activities expressed in nmol NADP reduced (or NADPH oxidised) per min per mg protein.

Whole cell lysates were prepared as in Table 2.

trolled by the internal potassium ion concentration⁸. In all cases where GS-GOGAT substantially contributes to glutamate synthesis, however, the change in amino acid pool during growth at low water activity is not principally in glutamic acid. The accumulation of proline does not require the accumulation of a neutralising cation. In those organisms which do accumulate proline, no change in K⁺ concentration can be observed during growth under osmotic stress. For example in *Bacillus subtilis* the intracellular K⁺ concentration is 250 mM irrespective of the external NaCl concentration. This does not, however, mean that any osmoregulatory mechanism cannot involve K⁺. After an osmotic shock, and before the subsequent accumulation of amino acid, water is removed from the cell by the osmotic pressure difference. Because the cell is largely impermeable to many intracellular solutes, such solutes will remain inside the cell after osmotic shock, and their effective concentrations will increase. K⁺, of course, is among these solutes. Once amino acid concentration has begun to increase within the cell, water is drawn back into the cell also, and the effective concentration of the other intracellular solutes will decrease again.

Table 4 Effect of K⁺ ions on GS-GOGAT activity

Organism	Activity* of enzymes in		
	0	250	500 mM K ⁺
<i>Escherichia coli</i>	<10	10	10
<i>Klebsiella aerogenes</i>	10	15	10
<i>Bacillus subtilis</i>	25	25	20
<i>Staphylococcus aureus</i>	30	30	20

*Assayed according to the method of Meers *et al.*¹⁴.

Activities expressed as nmol NADPH oxidised per min per mg protein.

Similar osmoregulatory mechanisms to those described above operate in aquatic invertebrates⁹. Further, proline accumulation is important in plants that have evolved to resist dehydration¹⁰. Until the evolution of homoeosmotic mechanisms in higher animals this primitive mechanism may have been widespread throughout the biological world from the simplest prokaryotes through to higher plants and invertebrate animals.

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Heterologous reattachment of regular arrays of glycoproteins on bacterial surfaces

REGULAR arrays of macromolecular subunits have been observed on the surfaces of a wide range of bacteria in electron micrographs of negatively stained or freeze-etched preparations^{1,2}. The regular patterns have mostly tetragonal or hexagonal symmetry and the centre-to-centre spacing between adjacent subunits varies from 6 to 15 nm. Chemical analyses of isolated subunits have shown that they are composed mainly of protein¹ or glycoprotein (unpublished) with molecular weights ranging from 65,000 to 150,000. Studies of a few bacteria have shown that the isolated subunits can assemble spontaneously to form regular arrays with the same dimensions as those seen in intact bacteria³. In appropriate conditions the subunits also reattach to the surfaces of cell walls from which they have been detached. Little is known about the biological function of these regular arrays of macromolecules on bacterial surfaces. That they cover the surface, leaving no gaps², suggests that they are important, for instance, in providing protection against adverse environmental conditions. Otherwise they would be expected to have been lost during evolution.

I have examined the ability of isolated subunits to reattach to cell surfaces to understand more fully the mechanisms involved in the development and maintenance of a two-dimensional array of macromolecules on a growing cell surface, and to obtain evidence for the suggestion that the assembly of these arrays is a dynamic process². Two strains of taxonomically closely related hyperthermophilic clostridia were used (grown at 65-70 °C), *Clostridium thermosaccharolyticum* and *C. thermohydrosulfuricum*⁴, which have tetragonal and hexagonal surface patterns, respectively (Fig. 1). Various treatments were used to remove the subunits from the surface of isolated cell walls and complete removal was only obtained with H-bond dis-

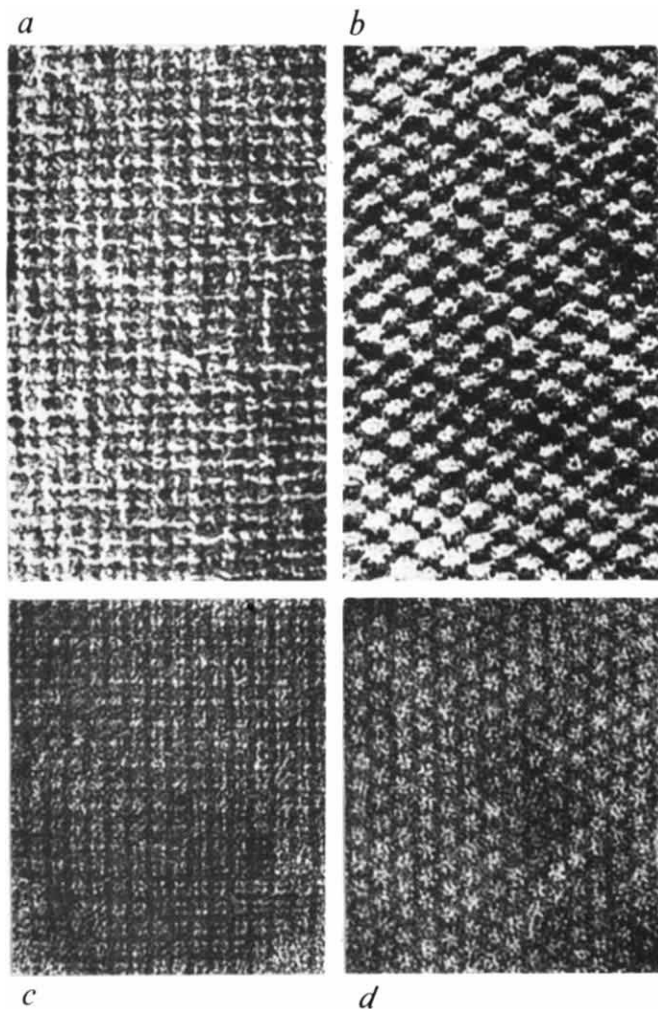


Fig. 1 Electron micrographs of freeze-etched preparations of the intact cells showing a portion of *a*, the regular tetragonal arrays of subunits on the surface of *C. thermosaccharolyticum* and *b*, the hexagonal arrays on *C. thermohydrosulfuricum*. ($\times 240,000$.) Electron micrographs of preparations of cell walls from *c*, *C. thermosaccharolyticum* and *d*, *C. thermohydrosulfuricum* negatively-stained with uranyl acetate. Cells were disrupted in an Aminco French pressure cell, and clean cell walls were isolated by treatment with Triton X-100 to remove the plasma membranes and washing in Tris-HCl (50 mM, pH 7.4) buffer. ($\times 240,000$.)

rupting agents, such as urea (8 M) and guanidine hydrochloride (GHCl, 5M). EDTA, Triton X-100, dithiothreitol and proteolytic enzymes, such as papain, trypsin, Pronase and thermolysin, had no visible effect on surface patterns. Analysis of treated cell walls and urea and GHCl extracts by sodium dodecyl sulphate (SDS)-polyacrylamide electrophoresis showed that the subunits of the surface layer form a major band which stains for both protein and polysaccharide (U.B.S., and K. J. I. Thorne, unpublished). The subunit protein of both microorganisms has a predominantly acidic amino acid composition and an acidic isoelectric point after isoelectric focusing on polyacrylamide gels. The molecular weights were 140,000, as determined by SDS-polyacrylamide electrophoresis.

Acidic conditions had an unexpected effect on the surface pattern. When cell walls were treated with buffer at a pH lower than 3, the regular arrays of subunits were no longer visible in negatively stained or freeze-etched preparations, and no periodicity was detectable in optical diffraction analyses of electron micrographs. No protein was lost, however, and the regular patterns reappeared very clearly when the pH was adjusted to neutral. Consequently it was con-

cluded that acidic conditions cause an uncoiling of the surface glycoprotein but do not detach it from the cell surface. When urea or GHCl extracts were dialysed against buffer at neutral pH (Tris-HCl, 50 mM, pH 7.4) the isolated subunits of both organisms could assemble into regular arrays *in vitro* in the absence of any supporting layer. This process was reversible, and the self-assemblies could be disintegrated and reformed by lowering and raising the pH, down to 2–3 and up again to 7. High salt concentrations (3 M KCl) had no effect on the self-assemblies. These chemical studies show that covalent bonds are not involved in the linkage between the subunits in the regular array, or between the subunits and the surface layer of the cell wall.

Isolated subunits reattach to cell walls in various conditions, such as dialysis of a mixture of urea or GHCl extract with 'naked' cell walls against distilled water or buffer. The reattached subunits form small areas of regular pattern, or crystallites, at various orientations (Fig. 2), and not the large regular arrays observed on intact cells. An unexpected finding was that subunits from one organism can attach to cell walls of the other organism and form patterns identical to those on the cells from which they originated. In addition, when cell walls from one organism are incubated with a mixture of both types of subunit, regular arrays, some with hexagonal and some with tetragonal symmetry, were observed on individual cell walls (Fig. 3). The two patterns were observed with equal frequency and the cell walls did not seem to favour the attachment of one type of subunit over the other. These observa-

Fig. 2 Negatively stained preparation of a fragment of cell wall from *C. thermohydrosulfuricum* which was incubated with subunits isolated from the same organism. The reattached subunits form small areas of hexagonal pattern. ($\times 120,000$.)



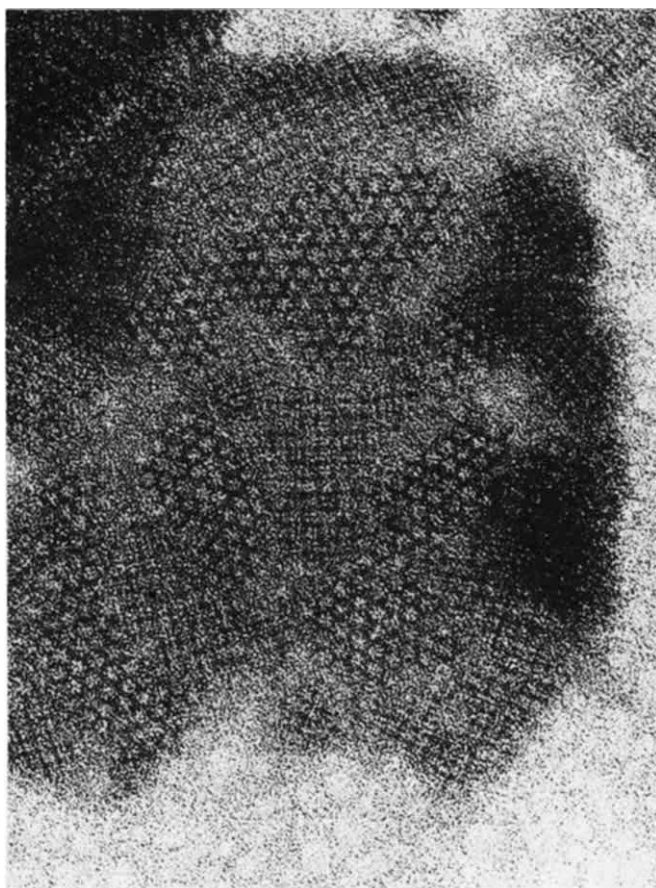


Fig. 3 Negatively stained preparation of a fragment of cell wall from *C. thermosaccharolyticum* which was incubated with a mixture of both types of isolated subunit. Small arrays of both hexagonal and tetragonal patterns are visible. ($\times 180,000$.)

tions clearly demonstrate that the information for the formation of the regular patterns resides in the subunits themselves, and is not affected by the supporting cell wall layer. In addition, the crystallites are orientated at random on the cell walls, suggesting that their orientation is not determined by any pattern in the binding sites in the cell wall.

An analysis of freeze-etched preparations of regular arrays of subunits on logarithmically growing cells of both clostridia, and other studies on thin sections (unpublished), have shown that large numbers of newly formed subunits appear at sites of division. These subunits first form a mosaic of small crystallites and subsequently rearrange themselves into large areas of uniform pattern during cell separation. From these observations it was concluded that the subunits undergo a dynamic process of assembly during cell growth². Such a process also requires a non-orientating supporting layer which allows free rotation and movement of subunits into the regular pattern. Thus it seems that the pattern is only determined by the directional bonds between the subunits and presumably represents the arrangement in which there is least strain and minimum energy in these bonds. The ability of the subunits to self-assemble into regular arrays and to adhere readily to the cell surface provides a simple mechanism for keeping a growing surface completely covered with a monolayer of macromolecules, providing that an excess of subunits is available either inside or outside the cell. The alternative mechanism of growth of a surface layer composed of regular arrays of macromolecules by the migration of dislocations³ is much more complex since it requires both the precise incorporation of subunits at particular points on the surface and that the rate

of glycoprotein synthesis be synchronised with the rate of synthesis of the underlying cell wall.

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Existence of a follicle-stimulating hormone inhibiting factor 'inhibin' in bull seminal plasma

THE existence of a substance of testicular origin capable of inhibiting the secretion of pituitary follicle-stimulating hormone (FSH) was postulated by McCullagh¹, who gave it the name 'inhibin', and direct, though not conclusive, evidence for its presence has since been provided²⁻⁶. After extraction and gel chromatography of bull seminal plasma, Franchimont *et al.*^{7,8} obtained a proteinaceous fraction (AcII) capable of decreasing basal FSH levels as well as diminishing the FSH response to exogenous luteinising hormone releasing hormone (LHRH) in normal and castrated adult male rats. We here summarise work based primarily on the specific biological effects of antiserum to AcII in rats, suggesting the existence of inhibin in bull seminal plasma.

Gel chromatography of bull semen (see Table 1) yielded a major unretarded peak (AcI: 200 ± 10 mg) followed by another distinct peak (AcII: 45 ± 5 mg) with a trailing edge. When 200- μ g aliquots of AcII were analysed by polyacrylamide gel electrophoresis⁹ (pH 8.6; gel concentration 10%), it was resolved into at least three components, as revealed by staining with Amido black.

AcI caused no significant decrease in serum FSH or LH in castrated adult male rats at a dose of 500 μ g per animal^{7,8}, whereas 200 μ g of AcII, intraperitoneally or intravenously, caused a consistent and significant decrease in serum FSH without affecting LH (Table 1). With normal intact males, a dose-dependent decrease in FSH was obtained after intraperitoneal injection (experiment 1, Table 1), although pituitary weight and gonadotrophin content were not affected.

The active fraction was examined for the possible presence of specific androgen binding protein (ABP) with high affinity for testosterone and dihydrotestosterone (DHT)¹⁰. Samples (100 and 200 μ g) were equilibrated at 0 °C for 2 h with tritiated testosterone, DHT and 17- β -oestradiol¹⁰. Proteins were fractionated by polyacrylamide gel electrophoresis and the radioactivity measured in 2.5-mm thick slices of the gel¹¹. No steroid-binding capacity for testosterone, DHT or 17- β -oestradiol could be detected in any of the three components of AcII. Thus, inhibin is neither identical nor similar to ABP elaborated by Sertoli cells under the influence of FSH¹⁰. The possibility that the biological effect of the active material was caused by contamination with steroids or their conjugates, was excluded by radioimmunoassay for testosterone, progesterone and 17- β -oestradiol after appropriate organic solvent extraction¹². None of these steroids

Table 1 Effect of seminal plasma fraction AcII on serum FSH and LH levels in adult male rats

Experiment	Treatment	Injection route	No. of rats	Serum FSH (ng ml ⁻¹ ± s.e.m.) NIAMD-FSH-RP ₁	Serum LH (ng ml ⁻¹ ± s.e.m.) NIAMD-LH-RP ₁
Normal rats	NaCl 0.9%	i.p.	10	581.0 ± 45.6	39.8 ± 7.1
	200 µg AcII	i.p.	10	423.0 ± 39.9*	39.1 ± 4.8
	500 µg AcII	i.p.	10	342.7 ± 44.6†	29.1 ± 4.6
	NaCl 0.9%	i.p.	5	638.0 ± 46	26.4 ± 5.3
	200 µg AcII	i.p.	5	458.0 ± 49.1*	20.0 ± 5.4
	NaCl 0.9%	i.v.	10	425.7 ± 30.8	48.9 ± 7.1
	160 µg AcII	i.v.	10	315.0 ± 32.2*	62.6 ± 8
	NaCl 0.9%	i.v.	10	1,261.0 ± 96	293.0 ± 25
Castrated rats	200 µg AcII	i.v.	10	855.0 ± 47.2†	320.0 ± 41.1
	500 µg AcII	i.v.	8	805.0 ± 31.4†	289.0 ± 45
	NaCl 0.9%	i.p.	10	1,080.0 ± 74.1	248.3 ± 61.7
	200 µg AcII	i.p.	10	795.0 ± 50.6*	324.2 ± 70.9
	NaCl 0.9%	i.p.	5	824.0 ± 83.9	421.0 ± 90.2
	200 µg AcII	i.p.	5	588.0 ± 56.2*	260.0 ± 22.7

Male albino Wistar rats (180–200 g) were given the total dose as four intraperitoneal (i.p.) injections at 12-h intervals. Rats were killed 4 h after the last injection, and blood and pituitaries collected. Alternatively, the test material or saline was administered as a single dose intravenously (i.v.) by way of the tail vein. Animals were autopsied 1 h later. Castrated animals were used 15 d after surgery. Serum samples were assayed for FSH and LH by radioimmunoassay using NIAMD systems¹⁶. Freshly collected bull semen was centrifuged at 4 °C to remove spermatozoa, and protein from the supernatant precipitated by addition of alcohol to a concentration of 86% (v/v). The precipitate was recovered by centrifugation in the cold, dissolved in distilled water and lyophilised. Batches of 300 mg were subjected to gel chromatography on Sephadex G100 in columns with a packed dimension of 2.5 × 30 cm, using 0.05 M acetate buffer pH 4.0 for equilibration as well as elution.

**P* < 0.05; †*P* < 0.025.

was detected in as much as 1,200 µg AcII. The possibility that the observed action of AcII may be a result of the presence of gonadotrophins, their fragments or metabolites, was also excluded by the fact that AcII does not cross react with either FSH or LH from man, sheep, rabbit or rat in the corresponding radioimmunoassay^{13–16}. An antiserum raised against AcII (see below) failed to bind labelled gonadotrophins of different species.

What seems to be significant is the demonstration that antibodies to inhibin can be raised in rabbits by active immunisation (2 mg AcII per animal in 1 ml saline mixed with an equal volume of Freund's complete adjuvant injected intradermally at several sites, followed by four booster intramuscular injections at 10–12-d intervals of 1 mg per animal in Freund's complete adjuvant; 15 d after the last injection the animals were bled by way of the carotid artery and sera recovered). The antiserum thus obtained is able to induce a marked increase in plasma FSH when injected into male or female rats.

The antiserum provoked a significant increase in the serum FSH levels in intact adult male rats when injected subcutaneously once a day for 4 d at doses of 0.5, 0.8 and 1.0 ml per animal, whereas serum LH, blood testosterone and testis and accessory organ weights were not significantly increased (Table 2). Nor did the antiserum lead to any histological changes in the seminiferous tubules.

In adult female rats, a dose of 1 ml antiserum per animal

Table 2 Effect of antiserum to seminal plasma fraction AcII on adult male rats

Treatment	No. of rats	Serum FSH (ng ml ⁻¹ ± s.e.m.) NIAMD-FSH-RP ₁	Serum LH (ng ml ⁻¹ ± s.e.m.) NIAMD-LH-RP ₁
Normal rabbit serum	25	320.7 ± 85.05	104 ± 69.5
0.2 ml Anti-AcII serum	5	306.0 ± 51.7	156 ± 76.3
0.4 ml	5	332.0 ± 66.4	121 ± 47.3
0.5 ml	5	958.8 ± 144.2*	209 ± 105.5
0.8 ml	5	1,570.0 ± 100.9*	187 ± 46.6
1 ml	5	1,420.0 ± 134*	290 ± 182.8

Antiserum to AcII was injected subcutaneously to adult male Wistar rats (180–200 g) once a day for 4 d, in graded doses (0.2–1.0 ml total dose) all made up to the same volume with saline; 12h after the last injection animals were killed for collection of blood, pituitary and reproductive organs. Pituitary and serum FSH and LH and serum testosterone were measured by radioimmunoassay.

**P* < 0.001.

Table 3 Effect of antiserum to seminal plasma fraction AcII on adult and immature female rats

Treatment	No. of rats	Serum FSH (ng ml ⁻¹ ± s.e.m.) NIAMD-FSH-RP ₁	Serum LH (ng ml ⁻¹ ± s.e.m.) NIAMD-LH-RP ₁
Adult rats			
Normal rabbit serum	5	203 ± 44.8	86.3 ± 34
Anti-AcII serum 1 ml per rat	5	3,120 ± 261.9†	715 ± 49.9*
Immature rats			
Normal rabbit serum, 0.5 ml per rat	6	330 ± 44.1	135.8 ± 33.1
Anti-AcII serum, 0.5 ml per rat	6	8,900 ± 517.9†	1,808 ± 291*

Adult (180–200 g) and immature (21–24-d-old) female Wistar rats were treated with a total dose of 1.0 or 0.5 ml antiserum to AcII according to the schedule described in Table 2. Pituitary and serum gonadotrophin and serum 17- β -oestradiol were measured by radioimmunoassay.

**P* < 0.005; †*P* < 0.001.

significantly elevated both FSH and LH levels without affecting serum 17- β -oestradiol, ovarian or uterine weights (Table 3). Administration of antiserum to immature female rats resulted in an even more pronounced elevation of both FSH and LH (Table 3). The antiserum had no effect on the pituitary gonadotrophin content in male or female rats. The FSH response to 1 ml antiserum was more striking in the adult female (1,536% control) than in the adult male (443% control), which could be a reflection of the different sensitivities of the male and female hypothalamo-hypophysial axis to inhibin. The apparent FSH specificity of responses to antiserum in male animals may be dose dependent: higher doses may also stimulate LH release in much the same way as small doses of LHRH selectively increase LH, whereas increasing doses stimulate the release of both FSH and LH in adult human subjects¹⁷.

Our observations, particularly those relating to the effect of antiserum to AcII, clearly demonstrate the existence in bull seminal plasma of a proteinaceous substance capable of diminishing circulating levels of gonadotrophins, especially FSH, in male and female rats. This substance, which corresponds with the definition of inhibin, may be important not only in the control of spermatogenesis but also of the oestrous cycle. In the light of the effects of the antiserum to AcII in immature females, the possibility

exists that this substance may also be important for the transition of females from sexual immaturity to adulthood.

Observations on the differential effects of normal and azoospermic human seminal plasma on FSH levels in adult, castrated rats² clearly implicate the germinal epithelium as the source of inhibin. Setchell and Sirinathsinghji³ pointed out that the logical route out of the testis for such a substance would be by way of the rete testis fluid (RTF) and the epididymis. Although a substantial part may be reabsorbed, together with RTF in the head of the epididymis, it would not be unusual to find this substance in the ejaculate. Viewed in this light, it is tempting to speculate that the source of inhibin in bull seminal plasma is indeed the germinal epithelium of the testis; however, we do not as yet have any evidence bearing directly on this possibility.

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Brain lesions and precocious puberty in rats

IN the immature female rat, the ability to secrete the gonadotrophins luteinising hormone (LH) and follicle-stimulating hormone (FSH) in response to a single injection of oestradiol or of progesterone first appears about day 28 of life^{1,2}. Younger animals respond to these hormonal stimuli with an increased release of gonadotrophins only if they have been primed with an injection of oestrogen 3 or more days earlier, and it is assumed that the priming steroid promotes the functional maturation of a positive (stimulatory) feedback system³ by an unknown mechanism. Alternatively, electrolytic lesions in the area of the basal hypothalamus of 23-d-old rats induce precocious sexual maturation leading to pubertal ovulation within 4 or 5 d (ref. 4). The lesion site is rich in luteinising hormone releasing hormone⁵, and it seems likely that the discharge of this polypeptide triggers the rapid and sustained increase in the production of ovarian steroids, particularly of oestrogen, observed during this period⁴. We report here that the increase of endogenous steroid in response to a brain stimulus can prime the positive feedback system in a manner closely related to the effect of exogenous oestrogen. This suggests that brain lesions advance the process of sexual maturation through the precocious establishment of a positive feedback loop.

Female Sprague-Dawley rats were kept on a 12/12 h light/dark cycle (lights on at 0700). On day 23 of life, at about noon, animals weighing > 56 g either received an injection of 10 µg oestradiol benzoate (OB) in oil (0.2 ml, subcutaneously) or

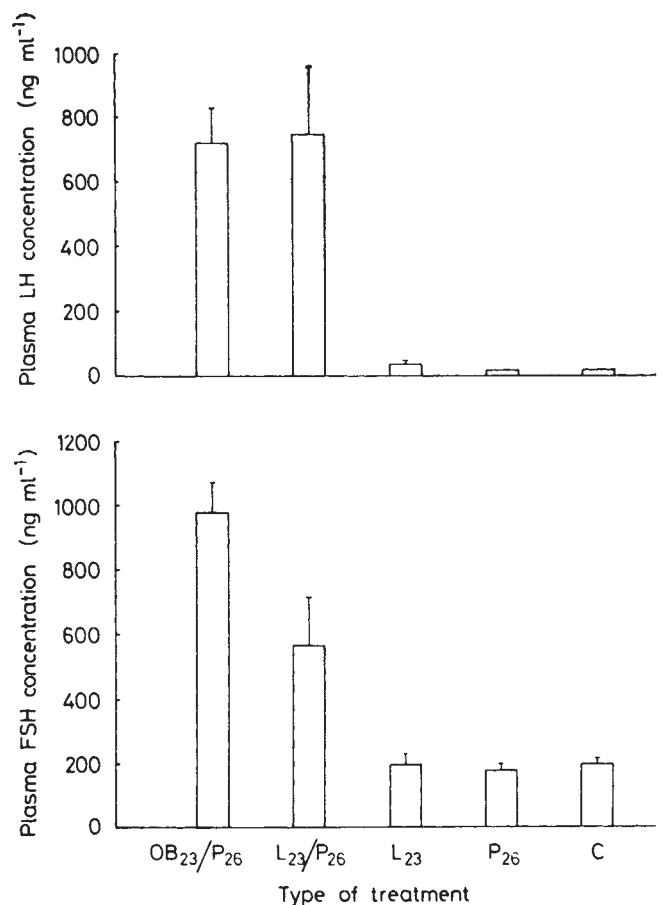


Fig. 1 Plasma LH and FSH concentrations 5 h after an injection of progesterone (P) in rats primed with oestradiol benzoate (OB) or with a brain lesion (L) and in controls. Samples were run in duplicate. Results are expressed as mean $\pm$ s.e. in terms of NIAMDD-rat LH-RP-1 and FSH-RP-1 reference preparations. Control values of LH falling below the detection limit of the assay were considered to be 15 ng ml⁻¹. C, untreated controls. Indices refer to age (in d) at which treatment was applied.

were subjected to a small unilateral electrolytic brain lesion placed stereotactically near the origin of the pituitary stalk⁴. Seventy-two hours later the rats received 1 mg of progesterone in oil (0.2 ml, subcutaneously); they were decapitated 5 h later (that is at 1700 on day 26). Plasma was assayed for LH and FSH concentrations using NIH radioimmunoassay kits. Controls were either untreated littermates of similar body weight, were subjected to brain lesions alone or were injected with progesterone only on day 26.

Figure 1 illustrates that both types of sequential treatment elevated mean plasma LH concentrations more than 20-fold compared with all types of controls ($P < 0.005$; Student's *t* test). FSH concentrations were significantly ($P < 0.05$) higher

Table 1 Degree of uterine hypertrophy induced by oestrogen/progesterone treatment or by sequence brain lesion/progesterone compared with controls

Group	n	Treatment	Uterine weights (mg; mean $\pm$ s.e.)	Significance (two-tailed <i>t</i> test)
1	8	OB ₂₃ /P ₂₆	150.9 $\pm$ 5.9	1 against 2, $P > 0.2$
2	8	L ₂₃ /P ₂₆	140.0 $\pm$ 12.6	
3	8	L ₂₃ only	104.5 $\pm$ 14.6	3 against 4, $P > 0.2$
4	8	OB ₂₃ only	121.3 $\pm$ 3.6	
5	8	P ₂₆ only	32.1 $\pm$ 1.9	
6	8	None	36.7 $\pm$ 5.5	

n, Number of animals per group. Rats were killed at 1700 on day 26, and uteri were dissected on filter paper. Indices as in Fig. 1.

after the combined hormonal treatment than after the sequence brain lesion/progesterone, but plasma values found after the latter approach nevertheless significantly ($P < 0.05$) exceeded control values in all groups. LH and FSH concentrations in controls were low, indicating that neither the brain lesion as such nor the injection of progesterone into unprimed animals can markedly increase plasma concentrations at this time.

The effect of the two treatments on uterine growth was compared (Table 1). The brain lesion (group 2) was found to substitute fully for the priming injection of OB (group 1) in this scheme; likewise, the degree of uterine hypertrophy induced by the lesion alone (group 3) was not significantly less than that seen after an injection of OB alone (group 4). In contrast, no uterine growth was induced when progesterone only was given on day 26 (group 5). From this cumulative bioassay, it is concluded that the brain lesion is equivalent to approximately 10 μg of injected OB.

The mode of action of brain lesions in the induction of precocious puberty is obscure⁶. It has been suggested that lesions eliminate the neural substrate which mediates the inhibitory action of gonadal steroids on the reproductive axis⁷. However, small unilateral lesions which spare most demonstrated oestrogen⁸ and progesterone⁹ uptake sites are efficient inducers of puberty⁴. Precocious sexual maturation can also be triggered, in rats, by repeated administration of oestrogen¹⁰ or by a single injection of pregnant mare serum gonadotrophin, and, in mice, by male-produced pheromones. The latter two mechanisms are dependent on the secretion of endogenous oestrogen^{11,12}. We submit that oestrogen also reinforces the stimulatory effect of the hypothalamic lesion.

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Rapid and dissociated changes in sensitivities of different dopamine receptors in mouse brain

DOPAMINERGIC systems in various parts of the mammalian brain, particularly in striatal, mesolimbic, mesocortical and hypothalamic structures, are involved in the control of motor activity, autonomic processes and emotional behaviour. An altered dopaminergic transmission in striatum seems to be responsible for Parkinsonism, whereas abnormalities in other, still poorly defined, dopaminergic synapses could participate in the aetiology of schizophrenia^{1–3}. Thus, there is considerable theoretical as well as therapeutical

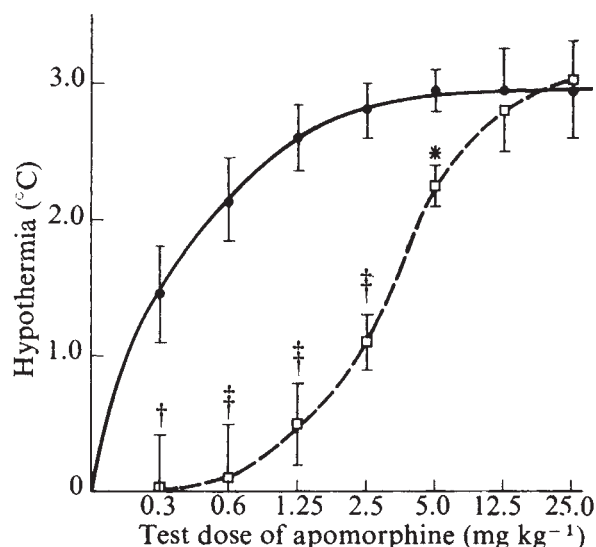
interest in defining specific characteristics of different dopaminergic systems participating in the control of these various functions.

Hypersensitivity to the action of specific dopamine agonists like apomorphine has been observed after either denervation⁴ or long term treatment with agents interrupting dopaminergic transmission^{5–7}, whereas desensitisation occurs after chronic stimulation⁸. The study of these states of modified receptor sensitivity may contribute to the understanding of the various factors controlling dopaminergic transmission in normal and pathological conditions and may also be helpful in defining several classes of dopaminergic synapses.

We observed that the sensitivity of dopamine receptors involved in thermoregulation and in a particular motor behaviour can be rapidly altered after a single administration of moderate dosage of an agonist (apomorphine) or an antagonist (haloperidol), respectively; however, whereas dopamine receptors controlling thermoregulation are easily desensitised and do not develop supersensitivity, the reverse is true for those implicated in the motor behaviour.

Male Swiss albino mice (22–26 g, Charles River, Saint Aubin-lès-Elbeuf, France) were housed in a well ventilated room, at 24 °C and under artificial illumination (light on between 0800 and 2000). Three hours before the experiments the animals were housed without food in small individual cages at 22 ± 1 °C. Body temperature was measured with a thermoelectric probe (Ellab RM 6, Copenhagen) inserted 4 cm into the rectum, immediately before and at various intervals after subcutaneous injection of apomorphine. Apomorphine induced an hypothermia which was related to dose both in intensity and in duration. The maximal hypothermia in controls (saline-pretreated mice) was observed at about 5 mg kg⁻¹ of apomorphine (Fig. 1) and lasted for about 90 min. In mice pretreated with this dose and tested after 8 h, there was a significant shift to the right of the dose-response curve: although the maximal hypothermia (–3 °C) was not modified, the ED₅₀ for apomorphine was 2.80 ± 0.59 mg kg⁻¹ as compared with 0.35 ± 0.06 in controls (calculated by the method of Miller and Tainter⁹). On the other hand, the hypothermia induced by agents, such as oxotremorine (0.05 mg kg⁻¹), clonidine

Fig. 1 Modification in the apomorphine-induced hypothermia after a single administration of the drug. Dose-response curves were established 8 h after the subcutaneous injection of either apomorphine (5 mg kg⁻¹) or saline. The effects of test doses of apomorphine were recorded 30 min after subcutaneous injection. Means $\pm$ s.e.m. of 10–15 experiments for each dose. Means were compared by Student's *t* test. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.



(0.25 mg kg⁻¹) or promethazine (10 mg kg⁻¹), acting on other systems, was not modified in the apomorphine-pretreated animals.

In mice treated with an antagonist of dopamine receptors, the antipsychotic drug haloperidol (0.1 mg kg⁻¹, intraperitoneally), the hypothermic action of apomorphine (0.45 mg kg⁻¹) was blocked for at least 10 h. After 72 h, the dose-response curve to apomorphine of mice pretreated with haloperidol (either 0.1 or 4 mg kg⁻¹) was not significantly different from that of controls. Similarly, the responsiveness to a single dose of apomorphine (0.30 mg kg⁻¹) was not altered 4, 5, 7 and 10 d after haloperidol (4 mg kg⁻¹).

In addition, a peculiar motor behaviour, that is climbing or verticalisation induced by apomorphine (refs 7, 10, 11 and P.P., J.C. and J.C.S., unpublished) was evaluated. For that purpose, after drug administration, the mice were immediately put into individual cylindrical cages, 12 cm diameter, 14 cm high, with walls of vertical metal bars, 2 mm diameter, 1 cm apart, surmounted by a smooth surface. After a 5-min period of exploratory behaviour, whereas the controls remained on the floor of the cage, the apomorphine-treated mice tended to adopt a vertical position holding the bars for at least 30 min, either with their forefeet only (small doses of apomorphine) or with all four paws (larger doses). This behaviour was scored as follows: four paws on the floor (0), forefeet holding the wall (1), four paws holding the wall (2). Two observations were performed on each animal, 10 and 20 min after injection of apomorphine and the two scores averaged. In controls, the maximal score was obtained for about 1.0 mg kg⁻¹ of apomorphine with an ED₅₀ of 0.50 ± 0.04 mg kg⁻¹ (Fig. 2). In mice pretreated with haloperidol (4 mg kg⁻¹, intraperitoneally) 72 h earlier, the dose-response curve to apomorphine was shifted significantly to the left (ED₅₀ = 0.27 ± 0.02 mg kg⁻¹).

Although pretreatment with apomorphine resulted in a diminished sensitivity to its hypothermic effect, the same was not true for the climbing behaviour. In fact, 8 h after administration of apomorphine (5 mg kg⁻¹) the ED₅₀ was 0.11 ± 0.02 mg kg⁻¹ as compared with 0.45 ± 0.04 for animals pretreated with saline in the same experiment. This

Fig. 2 Modification in the climbing behaviour induced by apomorphine after a single administration of haloperidol. Dose-response curves (10–30 experiments for each dose) were established 72 h after the intraperitoneal injection of either haloperidol (4 mg kg⁻¹) or saline. Means were compared by the χ^2 test ($\dagger P < 0.001$). □, Haloperidol-pretreated mice; ●, saline-pretreated controls.

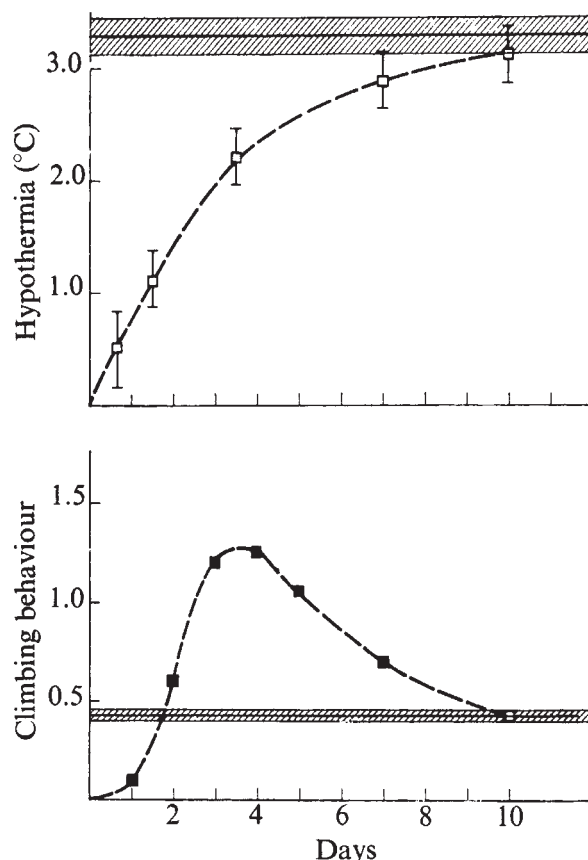
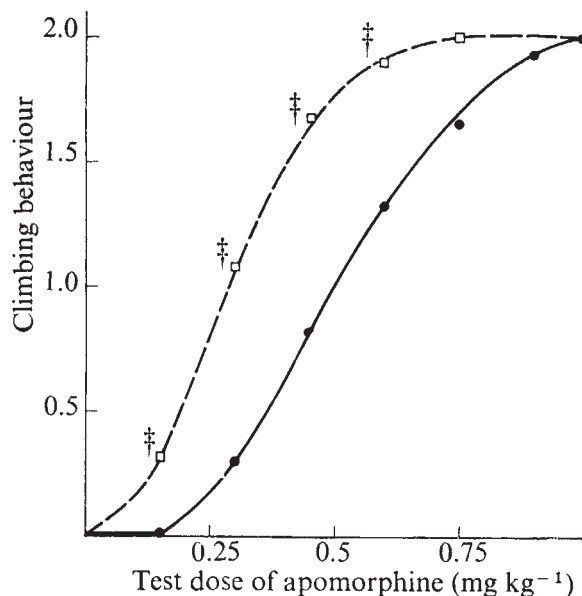


Fig. 3 Modification in the responses of mice to apomorphine at different times after a single pretreatment with either apomorphine (□, 5 mg kg⁻¹ subcutaneously) or haloperidol (■, 4 mg kg⁻¹ intraperitoneally). The intensity of the hypothermia in apomorphine-pretreated mice was determined 30 min after the subcutaneous injection of a test dose of 0.45 mg kg⁻¹ apomorphine (means of 15–30 experiments for each time interval). The climbing behaviour was scored after the subcutaneous injection of a test dose of apomorphine 0.30 mg kg⁻¹ (means of 10 experiments for each time interval). In both cases, the shaded area represents the mean ± s.e.m. of responses in saline-pretreated controls.

apparent facilitation of the climbing behaviour (which was also observed after a chronic treatment with apomorphine) could be due to the lack of hypothermia in these pretreated animals. This is suggested by a similar facilitation of the climbing behaviour, evaluated in naive animals, when they were kept at an environmental temperature of 30 °C instead of 22 °C, which compensates for the apomorphine-induced hypothermia.

Thus, it seems that a single administration of a drug acting at dopaminergic receptors is sufficient to modify the activity of apomorphine. It was therefore of interest to determine the onset and duration of the subsensitivity induced by apomorphine pretreatment and the hypersensitivity elicited by haloperidol (Fig. 3).

As early as 2 h after a dose of apomorphine (5 mg kg⁻¹, subcutaneously), when the normal temperature is reached again, a test dose of apomorphine (0.45 mg kg⁻¹) which, in controls decreased the temperature by 3 °C, had no effect. This refractoriness decayed progressively: it was still significant 4 d after treatment and completely disappeared only by 10 d. With regard to the climbing behaviour, the onset of the haloperidol-induced hypersensitivity could not be so precisely determined since the blockade by the antipsychotic lasted at least 24 h but there seemed to be no time lag between blockade and appearance of hypersensitivity (Fig. 3). Hypersensitivity was maximal between 72 and 96 h and also ceased by 10 d.

Modifications in the sensitivity of dopamine receptors

probably account for the modified responsiveness to apomorphine. The alkaloid is recognised as a specific agonist of dopamine receptors in the central nervous system on the basis of behavioural and biochemical evidence obtained in whole animal studies; in addition, the drug stimulates adenylate cyclase in striatal homogenates with a potency comparable with that of dopamine. The effects of apomorphine (including those two presently studied) are, like those of dopamine, antagonised by antipsychotics¹³.

It is unlikely that pretreatment with haloperidol or apomorphine interfered with the metabolism of the alkaloid since such a mechanism would have resulted in similar shifts in responsiveness in the two tests, which was obviously not the case. In addition, chronic pretreatment with haloperidol did not modify the apomorphine distribution in the brain⁷. It is also unlikely that the diminished hypothermic response to apomorphine resulted from a nonspecific compensatory adaptation of thermoregulatory mechanisms to the initial hypothermia since the response of other hypothermia-inducing agents like oxotremorine, clonidine, or promethazine, was not modified in apomorphine-pretreated mice. Also, subsensitivity to apomorphine developed as well when the hypothermia produced by the first treatment was prevented by keeping the mice in a hot environment.

Several studies have recently indicated that dopamine receptors in the CNS mediating various behaviours are either supersensitised by pharmacological interruption of synaptic function^{4,7} or, conversely, other receptors may be desensitised by sustained stimulation⁸. These results were, however, obtained by treatments extending over periods lasting from several days to several weeks. The striking feature in our experiments, which could bring new light to the process involved, was the rapid onset of the state of modified receptor sensitivity following the administration of a single dose of either the agonist or the antagonist. In fact in both cases there was no apparent time lag between the end of the drug effect and the appearance of this state of modified sensitivity, suggesting that the modifications take place while the drug is still acting on the receptor.

These events could be explained by postulating a conformational or functional change in the receptor as a direct result of drug combination or, more probably (as it is also observed after denervation), as an indirect consequence of the altered impulse activity in the postsynaptic neurone. The altered receptor would have either a different affinity for the agonist or a modified ability to initiate the characteristic responses: the shift in the dose-response curves, without alteration of the maximal responses (especially in the case of hypothermia which is clearly a gradual process) rather favours the first hypothesis.

It is interesting that the state of modified sensitivity had approximately the same duration in the two cases (that is, after hypersensitisation by haloperidol or desensitisation by apomorphine) which suggests that similar recovery processes are involved. The rather long duration of these states of modified sensitivity to apomorphine, which outlast by several days the drug action, may represent the time required either to replace non-reversibly modified receptors by newly-synthesised ones or, alternatively, to stabilise compensatory neuronal circuits.

The other interesting feature of the present observation is the easy desensitisation of dopamine receptors involved in thermoregulation which contrasts with their inability to exhibit hypersensitivity, although the opposite characterised those mediating the climbing behaviour, which could be hypersensitised but not desensitised. Reasons for this difference are not clear at present, but may be related to their location in different brain areas. The receptors mediating hypothermia seem to be localised in the mesolimbic system¹³ or, possibly, in the newly discovered dopaminergic system in the hypothalamus¹⁴. On the other hand, the climbing behaviour, which was suppressed by electrocoagulation

of the striatum and facilitated after denervation by 6-hydroxydopamine injected into this region (P.P., J.C. and J.C.S., unpublished), seems to be mediated by striatal receptors.

The exact relationship between the opposite changes in sensitivity and the location in different brain areas remains to be explored, however. One possible explanation could be that the first category of dopamine receptors (mediating the hypothermia) are, before any treatment, already in a state of maximal functional sensitivity whereas the reverse is true for the others, a difference which could result from the different activities in the respective presynaptic neurones. Other cerebral receptors, mediating the activation of cyclic AMP synthesis elicited by noradrenaline could be either desensitised¹⁵ or hypersensitised¹⁶.

Whatever their mechanism, the rapid, sustained and independent changes in sensitivities of different dopamine receptors in the brain may have important implications. It could provide an additional way to differentiate classes of dopaminergic synapses, a task not yet feasible by observing the short-term effects of drugs. It could be helpful in the development of animal models of diseases associated with altered dopaminergic transmission in the CNS. Finally, it could possibly offer a new therapeutic approach to these diseases.

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Release of ATP from stimulated nerve electroplaque junctions

THREE successive phases can be distinguished when the amplitude of the electrophysiological response of *Torpedo* electroplaques is recorded during stimulation at 5 or 10 Hz (ref. 1). A decrease, lasting about 30 s, is followed by a plateau of about 30 s during which the amplitude is maintained at 30-40% of its initial value. After the plateau, a late decrease brings the response to negligible levels in a few minutes. These three phases are correlated with variations in the tissue level of total acetylcholine (ACh)—the amount of transmitter decreasing during the first phase, increasing to control levels during the plateau and declining again. These variations are about 30-40% of the total level of ACh. No change is observed when only the pool of bound ACh is measured^{2,3}. In *Torpedo* homogenates, bound ACh (protected from hydrolysis by esterases³) is associated with a population of synaptic vesicles, which have been isolated by fractionation techniques⁴. It has been shown that ATP is

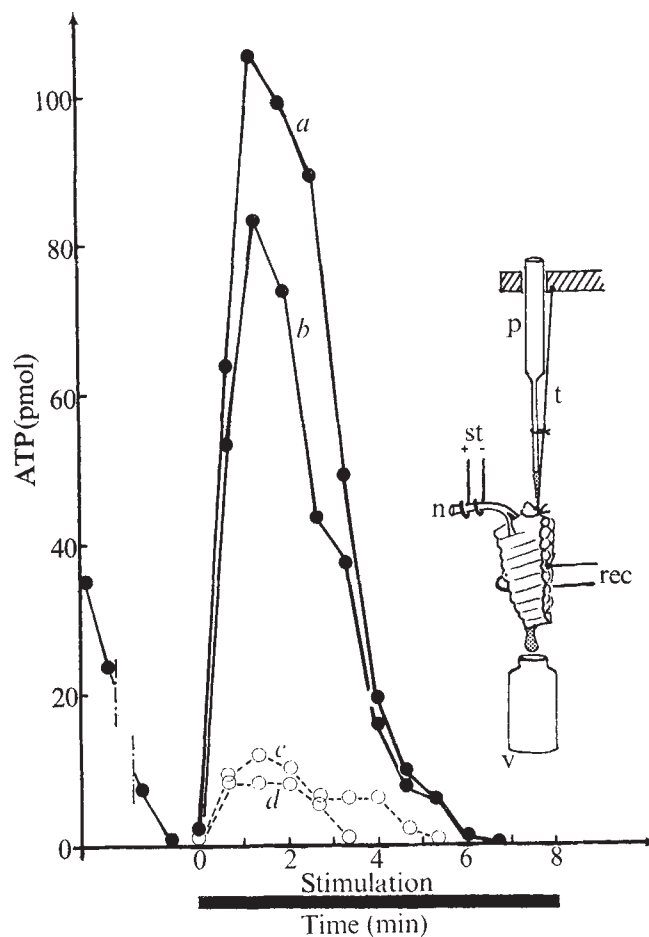


Fig. 1 Electric tissue and corresponding nerve. Perfusion fluid is brought by the Pasteur pipette (p), and the drops collected in the vial (V); a thread (t) holds the tissue; the nerve (n) is stimulated by the electrodes (St), and the response recorded by recording electrodes (Rec). Recording electrodes at each end of one prism. ●, Without curare; ○, with curare. *a* and *b*, Two experiments showing that ATP was collected in the perfusate when the nerve is stimulated. *c* and *d*, Curare inhibited the release of ATP. A section of electric organ (1 or 2 prisms thick) was dissected with its corresponding nerve, the fish being anaesthetised with Tricaine (1 g per 3 l seawater). The tissue was superfused with a physiological solution as described previously. The fluid (1 drop every 10 s) was collected in vials, in which the ATP assay was immediately carried out using the firefly luciferin-luciferase method. The Sigma-buffered firefly lantern extract was dissolved in 5 ml water and 100 μ l of the solution was added to the sample containing ATP. The light emission was measured using a liquid scintillation spectrometer SL30 intertechnique.

present in the vesicular fraction⁵. When *Torpedo* electric organ tissue is stimulated more than would be necessary to exhaust the electrophysiological response, or when for some other reason synthesis of new transmitter is rendered inefficient, a decrease in bound or vesicular ACh is observed. Of all the variations in ACh described, only the decline in bound ACh has been thoroughly investigated and confirmed by other laboratories⁶. It is known from fractionation experiments that both ATP and ACh are associated with synaptic vesicles, and a decrease in the vesicular content of the two substances was found in the experimental conditions that produce a decline in the pool of bound ACh (ref. 6). Several authors have collected ATP in the perfusate from stimulated neuromuscular junctions⁷⁻⁹, but the nucleotide was not found in perfusates from stimulated autonomic ganglia¹⁰.

As the electric organ of *Torpedo* is richly innervated and homologous to neuromuscular junctions, the preparation may be used to determine whether or not the nucleotide is

released, and if so from what structure such release occurs (for details of method, see Fig. 1).

Figure 1 shows that some ATP is released when the tissue is mounted, but this rapidly decreases to background levels. The stimulation (pulses of 1 ms duration at 5 Hz) was delivered to the nerve. A maximal initial response of 6–8 V was recorded and its decline followed during a 10-min stimulation period. Immediately after the onset of stimulation, ATP appears in the superfusate and its level falls when the electrophysiological response is exhausted. (Fig. 1*a* and *b*). In a similar experiment, carried out after incubating the tissue for 30–60 min in *d*-tubocurarine chloride 10^{-4} M, the electrophysiological response was reduced to a few 100 mV and the amount of ATP released on stimulation became negligible. (Fig. 1*c* and *d*). In similar experimental conditions using labelled precursors of ACh, we have shown that the release of transmitter was not affected by curare. If we assume that the only effect of curare is to protect the post-synaptic cell from the effects of ACh, these results suggest that ATP release during stimulation is a postsynaptic event. In previous work, it has also been shown that the tissue level of ATP declines during stimulation and that this effect is inhibited by curare¹¹.

Such ATP release may be considered as a postsynaptic event probably independent of the decrease in vesicular ATP occurring when the bound pool is utilised. We have also observed^{12,13} in-phase oscillations in the levels of ACh and ATP during stimulation. These fast oscillations (5-s period) are still not fully understood and may not necessarily be connected with the release of ACh from the nerve terminal or with postsynaptic ATP release. Moreover, it has previously been observed that addition of ATP to the incubation bath modifies the evolution of the electrophysiological response during stimulation (transient increase of the plateau)¹⁴. All these observations indicate clearly that ATP exerts its action at different levels during synaptic activity.

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Voltage clamp study on inward chloride currents of spherical muscle cells in tissue culture

EMBRYONIC chick skeletal muscle fibres grown in tissue culture generate three types of action potential different in their underlying ionic mechanisms and which are known accordingly as Na, Ca and Cl spikes¹. Because of their irregular and cylindrical shape^{2,3} these muscle fibres are not suitable for further analysis of the ionic mechanisms in using voltage clamp techniques. Voltage clamp techniques could however be applied successfully to those cultured muscle cells which, after incubation in a medium containing colchicine, had become spherical

myosacs²⁻⁶. In preliminary studies, the myosac generated Na, Ca and Cl spike components² and also, under voltage clamp, a late inward current (LIC) not necessarily due either to Na or to Ca (ref. 3). I now report a further voltage clamp study of the myosac revealed that the amplitude of the LIC was related to the concentration gradient of Cl ions across the cell membrane, and that the LIC was generated by a slow increase of Cl conductance which was dependent not only on the membrane potential, but also on the time course of the membrane depolarisation. Such Cl conductance provides the basis for the generation of a regenerative Cl spike in tissue-cultured skeletal muscle cells.

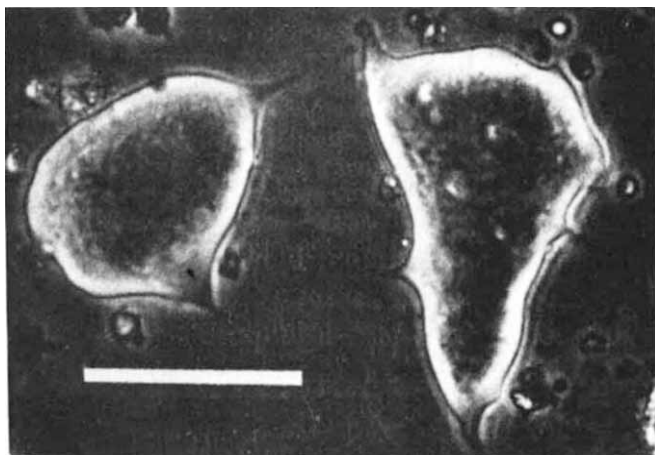


Fig. 1 Two myosacs on the 7th day in tissue culture. 10^{-8} M colchicine was added to the tissue culture medium during the 4th and 6th day of muscle development. Phase contrast microscope. Scale: 50 μ m.

Myoblasts (3×10^5 to 4×10^5 cells ml^{-1}) were obtained from pectoral muscles (dissociated mechanically) of 10–12-d old chick embryos and grown on a collagen-coated plastic dish (Falcon) in a modified Eagle's minimum essential medium⁷. Colchicine (10^{-8} M final concentration) was added to the medium during the 4th and 6th day of tissue culture, and the original long multinuclear myotubulus developed into independent myosacs 10–200 μ m in diameter (Fig. 1).

A myosac was penetrated with two glass microelectrodes (8 to 15 M Ω) filled with either 3 M KCl or 3 M K acetate solution. Membrane currents were measured with conventional voltage clamp techniques^{3,8}. To prevent possible inactivation of spike generation at low resting potentials² (-50 to -60 mV), the myosac was kept hyperpolarised to between -70 and -100 mV (holding membrane potential) by injecting steady currents of 2 to 10 nA (holding current). This holding current was also used for an injection of anions contained in the microelectrode into the myosac. The ionic composition in the bathing solution (NaCl 128.4 mM, KCl 5.0 mM, CaCl_2 5.0 mM and Tris-Cl 15.0 mM at pH 7.4) were altered under the voltage clamp condition.

Figure 2a–f illustrates the membrane currents generated by stepwise changes of the intracellular potential from the holding potential (-90 mV) to various levels. In this experiment, the microelectrodes were filled with 3 M K acetate solution. For small ranges of depolarisation or hyperpolarisation, the membrane currents consist of a transient capacitive current which lasts for a few ms (not visible in the figure) and a steady leakage current (Fig. 2e and f). When the membrane depolarisation exceeds a certain level (threshold), a LIC is seen superimposed on the two currents above. The LIC reaches its maximum amplitude of 2–5 nA in 1 to 10 s after depolarisation and decays thereafter to a level of the leakage current (Fig. 2d); the generation of the LIC is apparently time dependent. The same LICs can be generated repeatedly if the interval between the depolarisation is sufficiently long (usually 60–100 s). By increasing the depolarisation (Fig. 2d–a), the LIC becomes smaller in ampli-

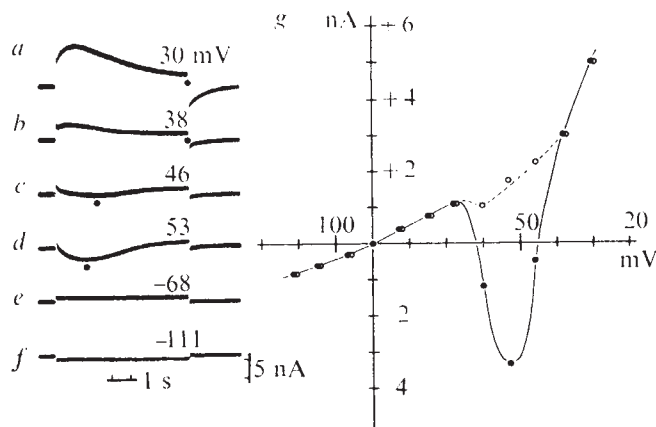


Fig. 2 Membrane currents and the I–V relationship examined under voltage clamp. a–f, Membrane currents elicited by instantaneous changes of the intracellular potential from the holding potential (-90 mV; holding current, 3.5 nA) to the levels indicated on each records. LIC is seen maximum amplitude in d. ●, Amplitudes of the peak inward currents or of the least outward currents (marked below records) during the depolarisation. ○, Amplitude of the currents measured at the end of the depolarisation. K acetate-filled microelectrodes were inserted into a myosac on the 5th day in tissue culture. See text for further details.

tude (Fig. 2c) and finally, most of the membrane currents become outward during a large depolarisation (Fig. 2a and b). It should be noted that shortly after the depolarisation, the myosac generated a transient inward current (50 to 200 nA) lasting 1–5 ms; this inward current, which was shown to be carried by Na ions³, is not seen in the figures of this paper because of the slow sweep velocity.

Figure 2g is an I–V relationship of the membrane currents obtained by plotting (1) the peak inward currents or the least outward currents and (2) the currents measured at the end of the depolarisation of Fig. 2a–g against the membrane potentials. The plots for the peak of the LICs form the N-shaped curve in Fig. 2g. This indicates that the LICs are generated as a result of an increase in the membrane conductance which represents the negative resistant part of the I–V curve and that this conductance increase is depended on the membrane potentials.

The LIC in Fig. 2 reaches its maximum amplitude between -50 and -60 mV. The maximum LIC was tested in the following ways. When Cl ions were injected into a myosac by means of a nearly steady current (holding current; see above) through a KCl-filled microelectrode, the amplitude of the LIC increased

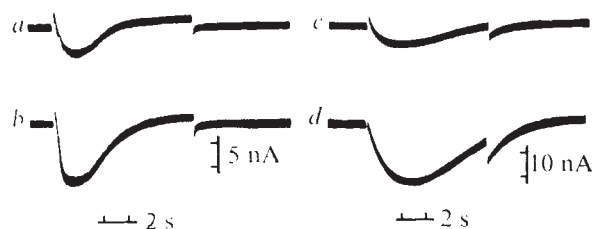


Fig. 3 Increase in amplitude of LICs associated with changes of Cl ion concentration. a–b, Intracellular injection of Cl ions by holding membrane current (about 4 nA) through a KCl-filled microelectrode caused a gradual increase in the amplitude of LICs elicited by the membrane depolarisation from -85 mV to -55 mV. a and b were recorded 5 and 11 min after the onset of the Cl injection, respectively. A myosac on the 6th day in tissue culture. c–d, Decrease of the extracellular Cl ion concentration (replaced by acetate ions) from 158.4 mM (c) to 15.0 mM (d) caused the increase in the amplitude of the LICs elicited by the membrane depolarisation from -75 mV to -56 mV. Before recording c, the myosac (on the 7th d in tissue culture) was loaded with Cl ions by 2 to 3 nA holding current for about 15 min, and the LICs became steady in amplitude. The solution change from c to d was performed in 3 min.

in time. This is shown in Fig. 3a and b by comparing the LIC elicited at about 11 min (b) with that at 5 min (a) after the onset of the Cl injection. In spite of the increase in amplitude, the time course of the membrane currents remained almost constant during the injection of Cl ions (Fig. 3a and b). Such an increase in amplitude of the LICs, however, did not occur (or rather decreased slightly) in the control experiments where acetate ions, instead of Cl ions, were injected intracellularly through a K acetate-filled microelectrode (not illustrated). Since acetate ions are considered impermeable through various Cl-permeable membranes⁹⁻¹¹, the increase in the amplitude of the LIC described above could be attributed to an elevation of Cl ion concentration in the intracellular space of the myosac.

The amplitude of the LIC became almost steady after about 10–15 min of the Cl-loading procedure. When Cl ion concentration in the bathing solution was decreased by replacing Cl ions with an equimolar solution of acetate (or propionate) ions, the LICs increased further in amplitude (Fig. 3c–d). But an alteration of the cation species in the bathing solution, such as Na (replaced by Tris), K (replaced by Na in the range 2–20 mM) or Ca (replaced by Na in the range 2–20 mM) ions, did not induce a significant increase in the amplitude of the LICs (not illustrated). These observations indicate that the amplitude of the LIC is related to both the intracellular and extracellular concentration of Cl ions, namely the concentration gradient of Cl ions across the myosac membrane.

It should be pointed out that the LICs were elicited under conditions in which the membrane potential was held at a hyperpolarised level below its resting membrane potential and the myosac was loaded with Cl ions. In these conditions, the electromotive force of the LICs, E_{Cl} , would be more positive than the holding membrane potential. An increase in the Cl conductance at the membrane potential depolarised below E_{Cl} would therefore generate an inward current³. It should also be mentioned that the net flux of the Cl ions during a LIC was directed outward.

The slow time course (that is, the peak time and duration) of the present LICs obtained in the voltage clamp experiments is compatible with the slow rise time and long duration of the Cl spikes elicited in the myosac under a current clamp condition. In fact, when a myosac was examined under the voltage clamp and current clamp conditions, the reversal potential of the LIC coincided closely to the peak membrane potential of the Cl spike³. These observations support the idea that the Cl conductance underlying the LIC is responsible for generation of the Cl spikes in cultured skeletal muscle fibres.

Inward Cl currents generated in *Nitella* and *Chara*¹²⁻¹⁴, and in electric organs of fish¹⁵ resemble the present LIC with respect to the time course and the dependency on the membrane potentials. Although the direction of currents is outward, a similar slow increase in Cl conductance has been reported in mature frog skeletal muscles^{16,17} and in sheep heart muscles¹⁸. The physiological role of the increase in the Cl conductance is not known for certain. Cl conductance may have a role in the coupling between the excitation and contraction¹⁹⁻²¹ or in the differentiation and maturation of muscle cells.

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Ionic permeability changes occurring at excitatory receptor membranes of chemical synapses

ALTHOUGH the ionic basis for generation of action potentials in nerve and muscle cells is now well understood¹, there is still much uncertainty about the changes in ionic permeability which result in the generation of graded depolarisations at receptor membranes of excitatory chemical synapses². The equilibrium potential for these graded depolarisations (E_R) is usually between -20 and $+10$ mV. This suggests an underlying increase in sodium permeability (P_{Na}) and potassium (P_K) and/or chloride (P_{Cl}) permeability, for in most nerve and muscle cells, the sodium equilibrium potential (E_{Na}) is very positive ($+50$ to $+75$ mV) and the potassium and chloride equilibrium potentials (E_K and E_{Cl}) are very negative (-40 to -100 mV). There is evidence that an increase in P_{Na} does occur during activation of excitatory synaptic membranes³, but there are few such membranes for which there is evidence for an increase in either P_K or P_{Cl} . Here we review and analyse the data obtained from previous investigations on the ionic basis of the graded depolarisations occurring at excitatory synapses. We demonstrate that the application of the Goldman-Hodgkin-Katz equation^{3,4} to previous data suggests that an increase in P_K as well as P_{Na} probably occurs at most if not all excitatory synapses.

At the vertebrate endplate, the transmitter substance and acetylcholine cause an increase in sodium and potassium conductance (Δg_{Na} and Δg_K), and

$$\frac{\Delta g_{Na}}{\Delta g_K} = \frac{E_K - E_R}{E_R - E_{Na}} \quad (1)$$

$\Delta g_{Na}/\Delta g_K$ remained constant during changes in external sodium concentration $[Na]_o$ and external potassium concentration $[K]_o$ between 5–150 mM and 0.25–7 mM respectively^{5,6}, with tenfold changes in $[Na]_o$ and $[K]_o$ within these concentration ranges causing shifts of E_R of about 30 and 25 mV respectively^{5,6} (Figs 1 and 2). Similar results were obtained at two other nicotinic cholinergic excitatory synapses, namely synapses generating the fast excitatory postsynaptic potentials in the frog sympathetic ganglion⁷, and the annelid neuromuscular junction⁸ (Figs 1 and 2).

For many other synapses, however, such as several cholinergic synapses in the molluscan central nervous system⁹⁻¹¹, glutamate synapses of insect^{12,13} and crustacean¹⁴ excitatory neuromuscular junctions, and mammalian smooth muscle cholinergic junctions¹⁵, E_R was altered by changes in $[Na]_o$, but not by changes in $[K]_o$ or $[Cl]_o$ (Figs 1 and 2). For example, in voltage clamp studies of the insect neuromuscular junction¹², E_R for the glutamate current and the excitatory postsynaptic current was $+3$ and $+4$ mV respectively in normal saline. In 0% NaCl, E_R for the glutamate current was $+8$ mV. E_R was not, however, altered when $[Cl]_o$ was decreased by 100% or when $[K]_o$ was changed between 0 and 20 mM. Consequently, it has been suggested that the synaptic current at all of

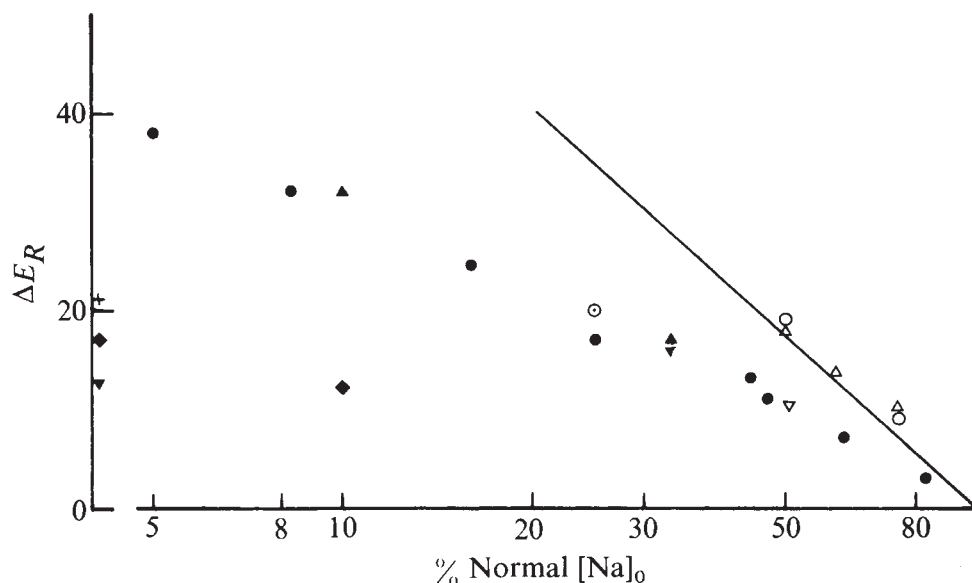


Fig. 1 Equilibrium potential (E_R) of the synaptic potentials and currents at different sites in different external concentrations of sodium, $[Na]_o$. The line represents a slope of 58 mV per tenfold change in $[Na]_o$. Most of the values of E_R are below both this 58 mV slope predicted by the Nernst equation if just Na was carrying the synaptic current, and also below the 50–57 mV slope predicted by the Goldman–Hodgkin–Katz equation if both Na and K were carrying the synaptic current (see Fig. 3). Abscissa: $[Na]_o$ (log scale). Ordinate: change in E_R . The data have been obtained from the following sources: ●^{3,6}, ○¹¹, △¹⁰, ▽¹⁴, ▽⁷, ○⁹, +⁸, ◆¹², ▲¹¹, ■¹³.

these sites was probably due exclusively to Na activation^{9–15}. There are also several other excitatory synaptic membranes at which it has been concluded that only Na carries the current during activation (for review see refs 2 and 16).

The 'membrane conductance' of an ion depends not only on the membrane permeability for that ion but also on the concentration and distribution of that ion on either side and within the membrane¹. Changes in $[Na]_o$ and $[K]_o$ would therefore be expected to affect the membrane conductance but not the membrane permeability of these ions. We have used the Goldman–Hodgkin–Katz equation to predict changes in E_R when $[Na]_o$, $[K]_o$ and $[Cl]_o$ are altered at the crustacean neuromuscular junction, the molluscan central nervous system and the squid giant synapse, using the data of previous investigators^{10,14,17}. These sites were chosen as they illustrate

in E_R predicted for a constant permeability increase to Na and K are very different from those predicted for a constant conductance increase to Na and K, but they do agree closely with the observed results at many excitatory synapses. We therefore propose that an increase in conductance to both Na and K does occur at excitatory synapses at which E_R lies between E_{Na} and E_K and at which altering $[K]_o$ between 0 and about 200% normal does not cause an observable shift in E_R . At the vertebrate endplate, E_R for the endplate potential at low temperatures (2 °C), and E_R for the action of suberythrine, a cholinomimetic agonist, are not changed when $[K]_o$ is lowered from 2.5 to 0.5 mM (ref. 18). It was therefore suggested that $\Delta P_{Na}/\Delta P_K$ does stay constant for these conditions when $[K]_o$ is lowered¹⁸.

Although there is much evidence that Na activation occurs

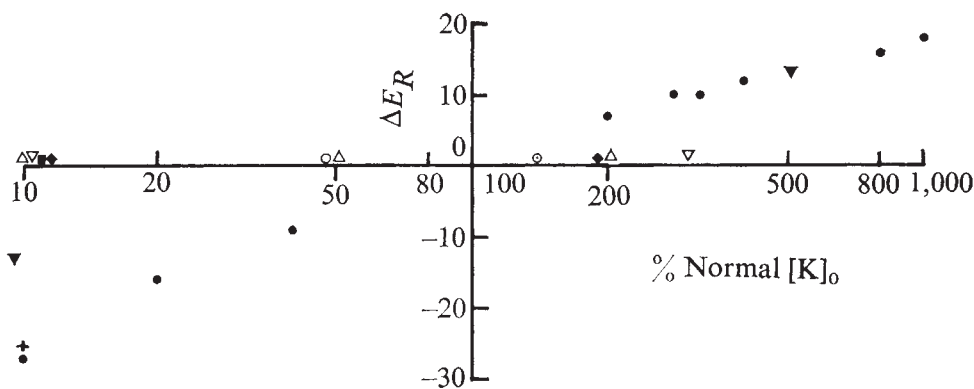


Fig. 2 Equilibrium potential (E_R) of the synaptic potentials and currents at different sites in different external concentrations of potassium, $[K]_o$. Abscissae, $[K]_o$ (log scale). Ordinates, change in E_R . References for symbols as in Fig. 1.

the predictions of the Goldman equation for a large range of permeability ratios and ionic concentrations. The analysis was carried out assuming that a permeability increase occurred to either Na and K (ΔP_{Na} and ΔP_K) or Na and Cl (ΔP_{Na} and ΔP_{Cl}). The values of $\Delta P_{Na}/\Delta P_K$ were calculated in normal saline from the equation:

$$E_R = \frac{RT}{F} \ln \frac{[K]_o + \Delta P_{Na}/\Delta P_K [Na]_o}{[K]_i + \Delta P_{Na}/\Delta P_K [Na]_i} \quad (2)$$

To calculate $\Delta P_{Na}/\Delta P_{Cl}$, the terms for K were replaced by those for Cl. $\Delta P_{Na}/\Delta P_K$ and $\Delta P_{Na}/\Delta P_{Cl}$ were then assumed to stay constant when $[Na]_o$, $[K]_o$ and $[Cl]_o$ were altered. It can be seen from Fig. 3 that the equation predicts that varying $[Na]_o$ and $[Cl]_o$ causes large changes in E_R , whereas varying $[K]_o$ causes little change in E_R . In fact, the theoretical curve for log $[K]_o$ against E_R has a maximum slope of only about 2 mV for $[K]_o$ between 0 and 200% normal. These changes

at the majority of chemical excitatory synapses, the changes in E_R caused by lowering $[Na]_o$ are often much less than those predicted by the Goldman–Hodgkin–Katz equation if Na and K activation occurred (Fig. 1). A possible explanation is that calcium, which usually has a very positive equilibrium potential, is carrying some of the inward current. There is evidence that the synaptic membrane at the vertebrate endplate¹⁹ and at the insect¹² and crustacean^{14,20} neuromuscular junction is slightly permeable to calcium. Alternatively, lowering $[Na]_o$ may cause a reduction in ΔP_K (ref. 20 and R.A. and P.N.R.U., unpublished).

In conclusion we believe that the graded depolarisations recorded at excitatory synapses which involve an increase in conductance normally involves an increase in both P_{Na} and P_K . In addition, an increase in P_{Ca} may occur especially in low $[Na]_o$. The sites that have been examined in sufficient detail can be divided into two categories. In the first category, which seems to include the nicotinic ACh synapses at the frog

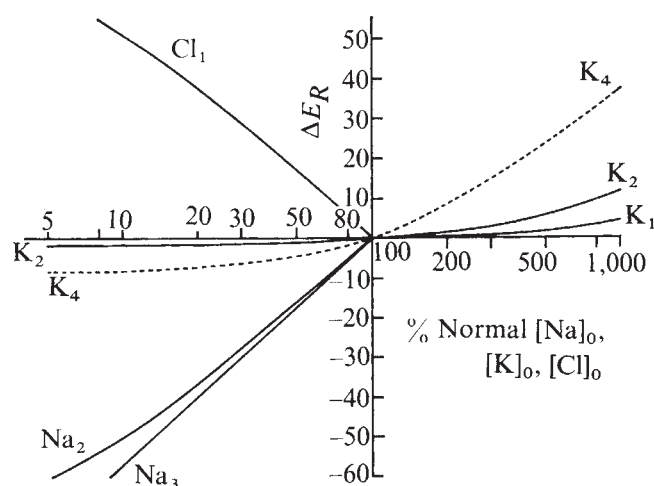


Fig. 3 Equilibrium potential (E_R) of the synaptic currents in different external concentrations of Na, K and Cl, as predicted by the Goldman-Hodgkin-Katz equation. The concentrations of ions and E_R in normal saline were taken from the data of previous investigations. (1) Crustacean neuromuscular junction¹⁴. (2) Molluscan central nervous system¹⁰. (3) Squid giant synapse¹⁷. (4) Crustacean neuromuscular junction (10 mM Na). The curves for Na_1 lie between Na_2 and Na_3 ; K_3 lies very close to the abscissae, and Cl_2 and Cl_3 are very close to Cl_1 . It can be seen that altering $[Na]_o$ and $[Cl]_o$ causes large changes in E_R , whereas altering $[K]_o$ causes little change in E_R , especially between 0 and 200% normal $[K]_o$, the range of concentration used by most investigators. In very low Na (10 mM), altering $[K]_o$ does cause much larger changes in $[K]_o$. The slope of the line relating $\log [Na]_o$ to E_R between 50% and 100% $[Na]_o$ is very similar for both the squid giant synapse and the molluscan CNS (57 mV and 52 mV respectively) in spite of $\Delta P_{Na}/\Delta P_K$ being 17.0 at the former and 0.3 at the latter.

and annelid neuromuscular junction and the frog sympathetic ganglion, the increase in conductance ratio to Na and K stays constant when the external ionic environment is modified. In the second category, which includes the acetylcholine synapses in the molluscan central nervous system, insect and crustacean glutamate synapses, and also probably the muscarinic acetylcholine synapses on mammalian smooth muscle, the increase in the ratio of Na conductance to K conductance changes when the external ionic environment is lowered, although the increase of the ratio of Na permeability to K permeability probably stays constant, especially during changes in $[K]_o$.

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Note added in proof: Ritchie and Fambrough²¹ have found that the acetylcholine reversal potential of cultured rat myotubes can be described by the Goldman equation in media of different $[K]_o$.

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Chemical modification of crab nerves can make them insensitive to the local anaesthetics tetrodotoxin and saxitoxin

CONDUCTION of the nervous impulse depends on voltage-sensitive change in the permeability of the nerve cell membrane to sodium ions. The sodium channels that appear during the action potential can be blocked very specifically by various neurotoxins, including tetrodotoxin (TTX) from the puffer fish and saxitoxin (STX) from certain marine algae¹. Shrager and Profera² presented evidence that the binding of TTX to crab nerves is reduced after treatment with a water-soluble carbodiimide and glycine methyl ester as the associated nucleophile. In these chemically modified nerves it follows that the sodium channels should have been rendered insensitive to TTX. Shrager and Profera were unable to test this prediction because in their experiments the nerves became inexcitable after chemical treatment.

We have carried out a similar modification and found conditions in which nerves continue to conduct impulses. Axons treated in such a way have a greatly reduced sensitivity to both TTX and STX, suggesting that either the sodium channel or its immediate environment has been modified such that the binding of both TTX and STX is markedly impaired without appreciably affecting the access of ions to the voltage-sensitive sodium channel.

In all our experiments, a water-soluble carbodiimide was used with various associated nucleophiles. Nucleophiles with pK_s in the neutral range and below were most satisfactory and these included diglycine, triglycine, glycine methyl ester, glycine ethyl ester, imidazole, semicarbazide, orthophosphate and *p*-amino benzene sulphonic acid.

Nerves were obtained from the walking legs of the spider crab (*Maia squinado*) and exposed to reagents in artificial seawater containing NaCl, 460 mM; MgCl₂, 55 mM; CaCl₂, 11 mM; KCl, 10 mM; NaHCO₃, 2.5 mM; together with 100 mM nucleophile and 100 mM carbodiimide at pH 5.5. After varying lengths of time the nerves were removed, washed well in normal artificial seawater (pH 7.8), and their sensitivity to TTX examined. In many instances treated nerves continued to conduct action potentials in the presence of 0.1 mM TTX, whereas untreated control nerves were blocked by TTX concentrations as low as 50-100 nM. To quantify this observation we used the conduction velocity technique of Colquhoun and Ritchie³. Nerves were mounted on a spaced set of platinum electrodes, and after the reaction, the conduction velocity was measured as a function of the external sodium concentration. In this way it is possible to equate inhibition produced by TTX to an equivalent reduction in external sodium and to obtain a rough estimate of the number of sodium channels that have been blocked, assuming $n=1$ in equation (3) of Colquhoun and Ritchie (Fig. 1).

Two features should be noted. The dose-response curve seems to be made up of two components: one with a normal high affinity for TTX, which presumably reflects unreacted sodium channels, and one with an affinity three

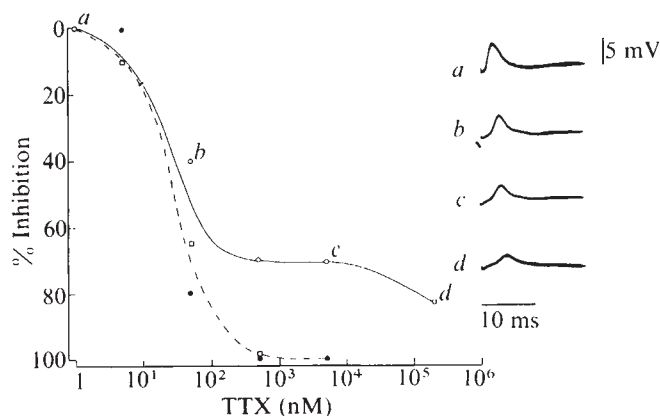


Fig. 1 Dose-response curves for blockage by TTX of sodium channels in crab nerve. Ordinate, reduction in number of functional sodium channels plotted as percentage inhibition; abscissa, concentration of TTX (nM) plotted on a log scale. ●, Control nerve immersed in artificial seawater (ASW); ○, nerve pretreated for 30 min in ASW containing 100 mM glycine methyl ester and 100 mM carbodiimide, pH 5.5 at 21 °C, compound action potentials corresponding to points *a*, *b*, *c*, *d* are shown on the right; □, nerve exposed to previous solution but in presence throughout of TTX (500 nM). After reaction, nerves were washed well in ASW before determining sensitivity to TTX. All nerves were obtained from the same animal.

to four orders of magnitude lower. These modified sites are partly inhibited in the presence of a saturated solution of TTX, but it is possible that at these concentrations TTX or an impurity is acting in a nonspecific manner. Exposure to carbodiimide and a suitable nucleophile while in the presence of a lethal concentration of TTX followed by repeated washing in TTX-free medium restored a conducted action potential that exhibits a sensitivity to TTX comparable with that seen in untreated controls. This observation shows that the presence of TTX protects against formation of the insensitive (or less sensitive) TTX-binding site.

To increase the proportion of sodium channels protected, we carried out the reaction in various conditions. As expected from the known chemistry of the carbodiimide reaction⁴ (Fig. 2), the rate of reaction increases as pH is reduced; but at low pH the nerve suffers irreversible damage even in the absence of reagents. We normally worked at pH 5.3–5.8. In these conditions the reaction rate increased linearly with increasing concentrations of either carbodiimide or glycine methyl ester. Some damage, evidenced by a reduction in conduction velocity, was seen in all preparations after exposure to the carbodiimide either alone or with a nucleophile. Glycine methyl ester alone had no effect on the preparation. Prolonged exposure to carbodiimide eventually blocked conduction, and the onset of this conduction block provided an upper limit to the exposure time. In optimum conditions (100 mM glycine methyl ester, 100 mM carbodiimide pH 5.5 for 30 min) we obtained modification of about 40% of the functional TTX-binding sites. The same treatment rendered a comparable percentage of sodium channels insensitive to another local anaesthetic, STX.

What group in the membrane is involved in generating the observed sensitivity to TTX? In aqueous solution the main groups known to react in the conditions of our experiments are thiols, phenols, amines, phosphates and carboxylates. In general, three reaction schemes are possible (Fig. 2).

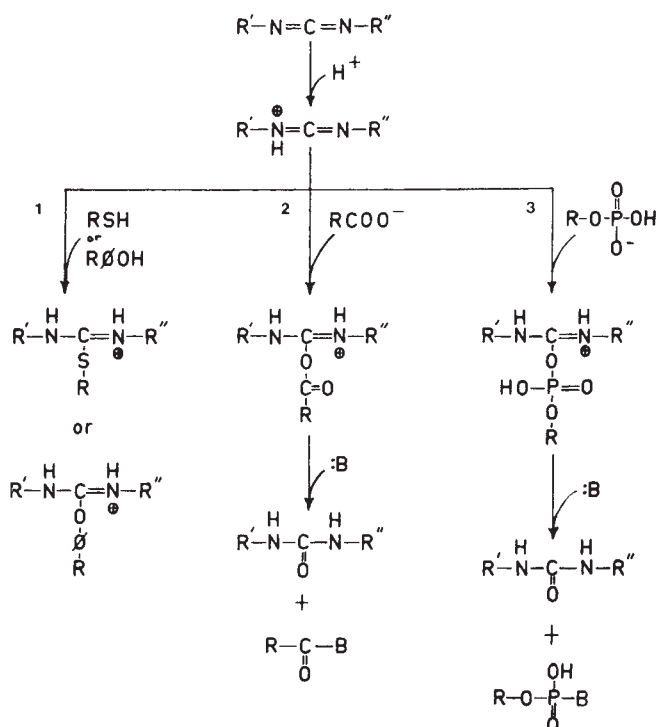
(1) The reactions with thiols, phenols and amines yield stable products without any added nucleophile⁵. It follows that reaction with carbodiimide alone should lead to a reduction in sensitivity to TTX, but this is never seen. Nerves exposed to carbodiimide alone slowly become in-

excitable, which may result from these reactions, or, conceivably, from reaction with a surface phosphate group either by effecting cyclisation of a phosphate with an adjacent *cis* hydroxyl or formation of a N-phosphorylurea^{4,6}. (2) The carbodiimide reaction with carboxyl groups yields an unstable intermediate which will react with a nucleophile. When the nucleophile is a primary amino compound the reaction should give a stable amide bond. Condensates with secondary amino compounds will be less stable. If, however, the nucleophile is orthophosphate, the product should be an acylphosphate which would be expected to hydrolyse at pH 5.5 with a halftime of less than one hour⁷. (3) With membrane phosphate groups, condensation with primary and secondary amines will form phosphoramidates; condensation with carboxyl will form acylphosphate; and condensation with orthophosphate will form pyro- or polyphosphates. Although the phosphoramidates are stable at pH 7 (refs 8–11), both acyl- and pyrophosphate are relatively easily hydrolysed^{7,12,13}.

There are problems in distinguishing experimentally between membrane-bound phosphate and carboxylate. A short-lived reduction in sensitivity to TTX (30 min) was seen after condensation with orthophosphate, which is consistent with both possibilities. In theory these possibilities might be distinguished if protection could be demonstrated after reaction with a carboxylic acid as nucleophile, because anhydrides are generally much less stable in aqueous solution than acylphosphates¹⁴. Condensation with carboxylic acids normally block conduction rapidly; but some protection against TTX was obtained with malonic acid, suggesting that a phosphate group may be involved. In addition, although not yet allowing further discrimination, we find that reaction with Meerwein's reagent (triethyloxonium tetrafluoroborate) also renders nerves insensitive to TTX.

The observation that TTX is able to protect against the chemical modification produced by carbodiimide and a suitable nucleophile raises the possibility that this might provide a convenient means of attaching a covalent label to the sodium channel. Thus, if a nerve is exposed to

Fig. 2 Possible reaction schemes involving membrane components, carbodiimide and nucleophiles. R, Unknown component of the membrane; R', ethyl; R'', dimethylaminopropyl; B, various nucleophiles.



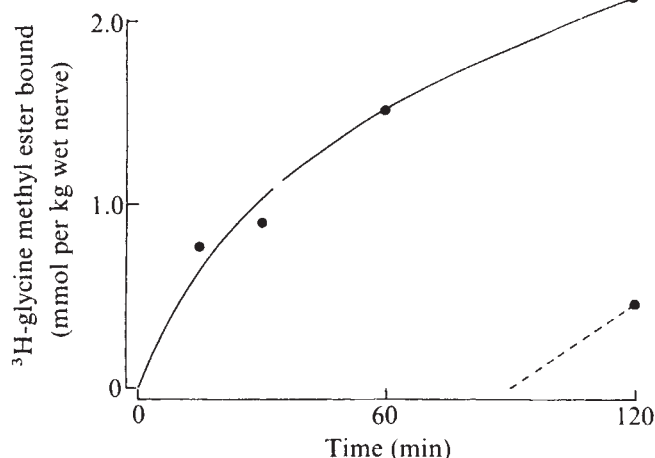


Fig. 3 Binding of ^3H -glycine methyl ester to crab nerve. Nerves were exposed to ASW containing 100 mM carbodiimide and 100 mM glycine methyl ester at pH 5.5. After incubation, nerves were washed well in ice-cold ASW containing non-radioactive glycine methyl ester, homogenised in distilled water to release any free intracellular label and centrifuged. The pellet was washed twice with distilled water containing 10 mM ester, dissolved in Nuclear Chicago Solvent and counted. Temperature 21°C . Uptake shown by dotted line was obtained after exposure of the nerve for 90 min to non-radioactive reaction mixture.

carbodiimide and glycine methyl ester in the presence of TTX, subsequent removal of the TTX and re-exposure to carbodiimide and labelled glycine methyl ester might result in specific labelling of the sites previously protected by the TTX. We tested this possibility by making the methyl ester of ^3H -glycine using Fischer's method¹⁵, and measuring the uptake of label after reaction with crab nerve in optimal conditions. Figure 3 shows that uptake continued for many hours and that considerable radioactivity became associated with the nerve. If the uptake is expressed in terms of the available nerve membrane area¹⁶, after 60 min the uptake is equivalent to 10^{14} labelled molecules per cm^2 or 10^6 per μm^2 or 1 molecule per $100 \text{ \AA}^2$. The number of sodium channels has been estimated to be about 100 per μm^2 (refs 1, 17, and 18) and it is perhaps not surprising that we were unable to detect any significant differences in the uptake of ^3H -glycine methyl ester between nerves pre-exposed to cold nucleophile in the presence or absence of TTX.

Labelled nerves provided further information on the nature of the covalent bonding. The majority of the label was stable to acid (1 h, pH 3.0), alkali (1 h, pH 10) and to neuraminidase; but was largely solubilised following exposure to a number of proteolytic enzymes including Pronase (Sigma Protease, Type V), trypsin and chymotrypsin, indicating that the label is mainly attached to proteins and polypeptides. The high density of these groups is somewhat unexpected although there is electrophysiological evidence for a similar number of negatively charged groups at the inner face of the squid axon membrane.

Our results show that a relatively straightforward chemical modification of the axon can lead to a dramatic change in its sensitivity to TTX and STX without affecting its voltage-sensitive permeability to sodium ions. This may help explain the relative insensitivity of some excitable cells to these toxins¹⁹. In its present form the carbodiimide-labelling technique produces too much nonspecific label to be of real use in isolating sodium channels; but it is probable that if the technique outlined here, or some modification of it, were applied to a partially purified preparation of TTX-binding sites it may be possible to attach a covalent label to the sodium channels that would aid purification of

these channels while still retaining their characteristic voltage-sensitive permeability to sodium ions.

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Direct evidence of importance of lysosomes in degradation of intracellular proteins

THE mechanisms of breakdown of intracellular proteins are largely unknown. It is widely presumed that lysosomes are important agents of this process¹, chiefly because they contain suitable enzymes². Although autophagic uptake and degradation of cytoplasmic organelles by lysosomes has been described³, there is only circumstantial evidence for significant lysosomal involvement in turnover of intracellular proteins³⁻⁶. Indeed, it is difficult to explain the heterogeneous turnover rates of proteins⁷ by a process involving bulk uptake of material¹. Furthermore, there is evidence for alternate systems of intracellular protein breakdown^{8,9}. In contrast, it has been shown that macrophage lysosomes are largely responsible for the degradation of endocytosed proteins, and that cathepsin D participates⁹. In the work reported here, breakdown of endogenous proteins of the perfused rat liver has been retarded by a specific inhibitor of cathepsin D, directed to the lysosomes, thus demonstrating their importance in the process.

The pentapeptide pepstatin¹⁰ is an inhibitor of carboxyl proteinases (termed acid proteinases by Hartley¹¹, because they generally have acid pH optima) such as cathepsin D¹². Cathepsin D, a lysosomal² enzyme with optimum activity near pH 5, adapted to the probably acidic lysosomal milieu, is the only known carboxyl proteinase in rat liver. Data on neutral proteinases^{1,2}, which might be capable of proteolysis in the cytoplasm, continue to appear. But with the exception of the specific kidney endopeptidase renin (which is now known to be a carboxyl proteinase¹³), none of these enzymes is inhibited by pepstatin. Thus pepstatin has no effect on autoprolysis of rat liver cytosol proteins at neutral pH⁶. The time course (up to 4 h) of digestion of a cytosol protein mixture⁶ at neutral pH (where cathepsin D is inactive) by sonicated rat liver homogenate (5 mg protein per ml) in the presence of 1% Triton X-100 has now also been found to be unchanged by pepstatin (0.1-10 $\mu\text{g ml}^{-1}$). Pepstatin is therefore suitable for the specific inhibition of rat liver cathepsin D.

Table 1 Rate of output of TCA-soluble radioactivity

Addition made at 105 min	Rate of output	
	% Initial rate	Average of group (% initial rate $\pm$ s.d. (n))
(a) None	97	100 $\pm$ 4 (3)
	105	
	99	
(b) 2 ml 5 mM potassium phosphate buffer, pH 7.0, containing pepstatin (50 μ g ml ⁻¹)	107	101 $\pm$ 6 (3)
	95	
	101	
(c) 2 ml control liposomes	81	93 $\pm$ 13 (3)
	108	
	90	
(d) 2 ml liposomes containing pepstatin (50 μ g ml ⁻¹)	32	47 $\pm$ 17 (3)
	66	
	44	

Perfusions and additions were as described for Fig. 1. Rats were 250–300 g body weight. Initial rates of output were between 2.6 and 4.7 d.p.m. per ml medium per min, and corresponded to the release of 2–3% of initial liver protein radioactivity per hour. Correlation coefficients for the lines for perfusions in the absence of liposomes or in the presence of control liposomes were between 0.94 and 0.99. Those for periods after treatment with liposomes containing pepstatin were lower (0.87–0.94), probably because inhibition was not immediate. Leakage of malate dehydrogenase during the perfusion was always less than 3%, confirming the integrity of the livers. Livers receiving entrapped or free pepstatin were further perfused with a single pass of 0.9% NaCl (100 ml) at 130 min, and then homogenised in 5 volumes of 0.25 M sucrose 50 mM potassium phosphate buffer, pH 7.5. Inhibition of cathepsin D in the homogenates was estimated by assaying aliquots before and after dialysis against 50 volumes of phosphate buffer, pH 7.5, with one change. At pH above 6.4, there is little binding of pepstatin to cathepsin D (C. G. Knight, personal communication) and thus dialysis allows recovery of activity. The enzyme in homogenates of livers receiving liposomal pepstatin was approximately 80% inhibited, and that from livers receiving free pepstatin was approximately 30% inhibited. These values probably overestimate the degree of inhibition achieved in the perfusion since homogenisation will bring all uncomplexed inhibitor into contact with enzyme (as indicated by the inhibition of homogenate enzyme observed after treatment with free pepstatin, in the absence of inhibition of protein degradation). The percentage rates produced by treatments (a)–(c) do not differ significantly from 100%, whereas those following (d) are significantly different from those following (c) ($P < 0.05$).

Furthermore, pepstatin seems not to permeate macrophage plasma membranes (personal communication from A. J. Barrett and C. G. Knight) or other cellular membranes (with the possible exception of those of erythrocytes infected with malarial parasites¹⁴). Therefore it probably does not permeate lysosomal membranes, as might be expected for a pentapeptide¹. Because of this impermeability it was possible to direct the pepstatin specifically to lysosomes by entrapping it in multilamellar liposomes¹⁵ which are known to enter liver lysosomes rapidly after intravenous injection¹⁶. After endocytosis by Kupffer and parenchymal cells¹⁶, secondary heterolysosomes containing liposomes are formed, and by subsequent fusions, the liposomes also reach autophagic secondary lysosomes¹⁶. This method of administration further reduces the possibility of inhibition of unknown carboxyl proteinases outside the lysosome, by limiting access of the inhibitor to the cytoplasm.

It has been shown⁴ that livers of rats prelabelled with ¹⁴C-valine (given 2–4 h previously) lose labelled valine to the medium on subsequent perfusion. During perfusion in the presence of 15 mM cold valine (to prevent reincorporation) the linear rate of output of radioactivity is proportional to the rate of proteolysis in the period from 60 to 180 min after commencement. Radioactive secretory proteins have left the liver, and been taken up again to a negligible extent⁴ (my unpublished work). The method therefore measures the rate of degradation of intracellular proteins. I have determined this rate during

a 45-min period, and at 105 min from the start of perfusion, added liposomes containing pepstatin, or control liposomes, or free pepstatin, to the medium, and measured the subsequent rate. Figure 1 shows the results of a pair of such perfusions, and Table 1 summarises the results.

In perfusions to which control liposomes or free pepstatin were added at 105 min, there was no significant difference between the rates before and after the addition (Table 1). In contrast, there was a highly significant inhibition by liposomes containing pepstatin (Fig. 1, Table 1). The lack of inhibition by free pepstatin (Table 1b) supports the suggestion that pepstatin does not permeate liver cell membranes.

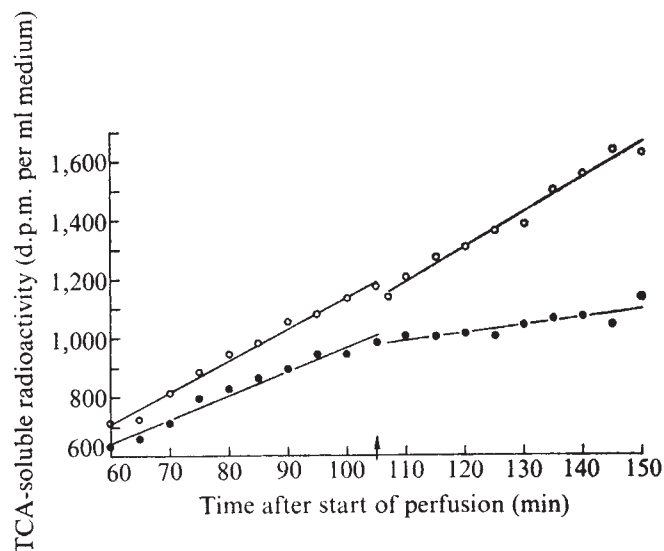


Fig. 1 Livers from rats prelabelled (2.5–3.5 h) with L-¹⁴C-valine (25 μ Ci) were perfused by a minor modification (addition of amino acids to a quarter of their concentration in DMEM, Flow Laboratories, Irvine, Scotland, and glucose, 14 mM, to the medium) of an established technique¹⁷. Medium radioactivity soluble in 5% trichloroacetic acid–10 mM valine (cold) was determined in samples (0.5 ml) taken every 5 min. The arrow indicates the addition of cationic liposomes (20 μ mol phosphatidyl choline, 20 μ mol cholesterol and 10 μ mol stearylamine, in 2 ml 5 mM potassium phosphate buffer, pH 7.0) which probably maintain a fairly constant intrahepatic concentration after a short time¹⁸, and which may reach parenchymal¹⁶ in addition to Kupffer cells. To prepare liposomes¹⁵, lipids (in chloroform-methanol, 2:1) were dried on to the surface of a glass homogeniser in a nitrogen stream, and desiccated under vacuum. After homogenisation (ten passes of a tight pestle) in buffer with pepstatin, 50 μ g ml⁻¹ (●; see also Table 1d) or in buffer alone (○; Table 1c), they were sonicated for 10 s, and used without further treatment. Entrapment of 20–30% of the pepstatin was demonstrated by gel filtration on Sepharose 4B in 0.9% NaCl, which separates free pepstatin from liposomes. Pepstatin was estimated¹² by measuring the activity of various known amounts of human cathepsin D in the presence of column fractions treated with 0.1% Triton X-100 to lyse liposomes. Latency of liposomal pepstatin was confirmed by refiltering entrapped pepstatin after lysis. Because a similar degree of capture of the anionic pepstatin was also observed with anionic liposomes (10 μ mol phosphatidic acid in place of stearylamine) its association with liposomes was probably not merely electrostatic. Line fitting was done by the method of least squares.

These results constitute the first direct evidence that degradation of intracellular proteins in liver is to a large extent performed by lysosomes. They also re-emphasise the role of cathepsin D in digestion of complex substrates, complementing work on cathepsin B₁ and other proteinases⁵. Although perfusion itself increases intracellular proteolysis⁴, the inhibition by pepstatin is great enough to indicate inhibition of both basal and stimulated proteolysis.

It seems probable that entry of proteins into lysosomes is normally the rate limiting step in lysosomal protein breakdown^{1,5}, particularly in view of the lack of evidence for escape of proteins from lysosomes. In the presence of inhibitors of lysosomal

proteinases, intralysosomal proteolysis is retarded, either leading to an intralysosomal accumulation of protein or perhaps to a feedback inhibition of uptake. Either of these events may occur also when lysosomal proteinases are genetically defective; that this has not been detected in man¹⁹ implies they are lethal in early embryonic life. The mechanisms of uptake, and their control and specificity, now deserve considerable further attention⁶.

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Synthetic substrate for cyclic AMP-dependent protein kinase

Most hormones and neurotransmitters are thought to stimulate the synthesis of adenosine 3',5'-cyclic monophosphate (cyclic AMP) which in turn activates protein kinases¹. On activation, the protein kinases transfer the terminal phosphate group of ATP to serine or threonine residues in enzymic or membrane proteins involved in metabolic regulation, thereby either activating or inactivating them. Only a few key proteins are phosphorylated^{1,2} which raises the question of the molecular basis for the recognition of a particular serine by the cyclic AMP-dependent protein kinases. Cohen *et al.*² considered this problem with respect to the phosphorylation of phosphorylase kinase and glycogen synthetase and concluded that the cyclic AMP-dependent protein kinase recognises some specific three-dimensional configuration formed by a particular amino acid sequence at the site of phosphorylation. The features of this structure, however, remain to be defined. It has been shown³ that small peptides from myelin basic protein could act as substrates, thus reducing the number of parameters for consideration.

A major site of phosphorylation by cyclic AMP-dependent protein kinase in intact myelin⁴, isolated myelin basic protein⁵, and in peptic peptides⁶ was serine 110 in the sequence Gly-Arg-Gly-Leu-Ser-Leu. Here we examine the phosphorylation of a synthetic peptide equivalent to amino acid residues 106-113 in the basic protein of human myelin⁷. The peptide is thus the first synthetic substrate for the cyclic AMP-dependent protein kinases which seem to be so important in regulatory processes.

The peptide Gly-Arg-Gly-Leu-Ser-Leu-Ser-Arg was synthesised by the solid phase technique⁸, as modified for auto-

matic synthesis (M. D. Geier, F. S. Geier, and J.D.Y., unpublished). The double coupling programme included chloroform as solvent, dicyclohexylcarbodiimide as coupling agent, 25% trifluoroacetic acid to remove *t*-butoxycarbonyl protecting groups and anhydrous HF at 0 °C for 30 min to cleave the peptide from the resin and to remove the O-benzyl and nitro groups from serine and arginine respectively. Initial purification was on Sephadex G-15 and the yield was 20%. The peptide was stained with ninhydrin and had a mobility of +0.59 relative to aspartic acid⁹ on high-voltage electrophoresis using Whatman 3MM paper in pyridine-acetate buffer, pH 6.5. Two minor contaminants with relative mobilities of +0.34 and +0.65 were removed by this technique. (These contaminants, which also acted as substrates for the protein kinase, presumably arose from the incomplete removal of the nitro-protecting group on the C-terminal arginine¹⁰.) The purified peptide on analysis contained equimolar ratios of its four constituent amino acids.

Protein kinases were prepared from bovine cardiac muscle and brain^{3,11} and further purified by gel filtration on Sepharose 4B in 5 mM potassium phosphate buffer, pH 7.0, containing 2 mM EDTA. The kinases were assayed with histone and with myelin basic protein as substrates and cyclic AMP produced a stimulation between four- and sevenfold.

In the experiments with synthetic peptide, the electrophoretic assay for protein kinase activity was used³. The actual incubation conditions are described in Fig. 1 and below. The peptide (250 nmol) was incubated with γ -³²P-ATP (1 μ mol, 8.7×10^6 c.p.m.), cardiac protein kinase (8.5 μ g), 4 nmol cyclic AMP, 1.4 μ mol magnesium acetate, 120 nmol EGTA, in 30 mM sodium acetate buffer, pH 6.5, in a total volume of 350 μ l at 30 °C for 2 h. The mixture was fractionated by preparative paper electrophoresis as described above and the paper sheet

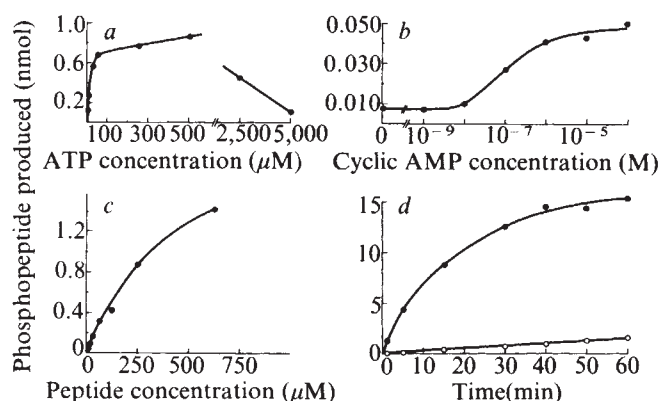


Fig. 1 Production of phosphorylated peptide Gly-Arg-Gly-Leu-Ser(P)-Leu-Ser-Arg; ordinate refers to the total nmol of phosphopeptide produced at 30 °C in each incubation tube in 5 min or, in *d*, for the time indicated. In *a*, *b* and *c* 10 μ l of each of the solutions (i), (ii) and (iii) were incubated in separate tubes with 10 μ l of cardiac protein kinase (85 μ g ml⁻¹). Incorporation of ³²P into the peptide was assayed on 25 μ l samples by the electrophoretic method³. *a*, Effect of ATP: (i) peptide in water (10 nmol per 10 μ l); (ii) 14 mM magnesium acetate, 1.2 mM EGTA and 40 μ M cyclic AMP, adjusted to pH 6.5, and (iii) γ -³²P-ATP (3.0×10^5 c.p.m. per 10 μ l) mixed with ATP to give the final concentration indicated in 100 mM sodium acetate buffer, pH 6.5. *b*, Effect of cyclic AMP: (i) peptide in water (3.1 nmol per 10 μ l); (ii) as in *a* but incorporating cyclic AMP to give the final concentration indicated; and (iii) 277 μ M γ -³²P-ATP (2.5×10^5 c.p.m. per 10 μ l) in buffer as in *a*. *c*, Effect of peptide: (i) peptide in water to give the final concentration indicated; (ii) as in *a*; and (iii) 2 mM γ -³²P-ATP (3.0×10^5 c.p.m. per 10 μ l) in buffer as in *a*. *d*, Effect of time and cyclic AMP. Two tubes were prepared with the following solutions (i) 5 μ l peptide in water (50 nmol per 10 μ l); (ii) 50 μ l as in *a* or in the second tube as in *a* but without cyclic AMP present; (iii) 50 μ l 2 mM γ -³²P-ATP (1.4×10^6 c.p.m. 50 μ l) in buffer as in *a*; and (iv) 50 μ l cardiac protein kinase. At times indicated 20 μ l were removed for assay.

Table 1 Sites of phosphorylation by cyclic AMP-dependant protein kinases

Substrate		Sequence	Sources of kinase	Ref.
Non-globular substrates				
Histone F1		Arg-Arg-Lys-Ala-Ser-Gly-Pro *	Liver	22, 23†
Histone F2b		Ser-Arg-Lys-Glu-Ser-Tyr-Ser *	Lymphocytes	24
Myelin basic protein	Site 1	Gly-Arg-Gly-Leu-Ser-Leu-Ser *	Muscle	
	Site 2	Gln-Arg-His-Gly-Ser-Lys-Tyr *	and	
	Site 3	Arg-His-Arg-Asp-Thr-Gly-Ile *	Brain	4, 5, 6
RCMM Lysozyme†	Site 1	Tyr-Arg-Gly-Tyr-Ser-Leu-Gly *		
	Site 2	Arg-Asn-Thr-Asp-Gly-Ser-Thr-Asp *	Muscle	21
Protamine	Site 1	Arg-Arg-Arg-Ser-Ser-Ser-Arg * * *		
	Site 2	Ala-Arg-Arg-Val-Ser-Arg-Arg *	Testis	25, 26
Globular substrates				
Phosphorylase kinase	α subunit	Arg-Leu-Ser-Ile-Ser Lys		
	β subunit	or-Gln-Ser-Gly-Ser-Val Arg or-Tyr Ile	Muscle	2
Troponin I	Site 1	Val-Arg-Met-Ser-Ala-Asp *		
	Site 2	Ser-Val-Met *	Muscle	18, 19
Pyruvate kinase (Liver)		Leu-Arg-Arg-Ala-Ser-Leu *	Liver	17
Glycogen synthetase		Lys or-Gln-Ile-Ser-Val Arg	Muscle	27

*Amino acid residue phosphorylated.

†RCMM Lysozyme, reduced carboxymethylated maleylated lysozyme.

‡S. C. Rall and R. D. Cole, in ref. 23.

subjected to autoradiography. A zone of radioactivity with a relative mobility of $+0.19$ was detected. A sample of this zone stained the typical yellow colour given by glycyl peptides. The zone was eluted with $0.01\text{ M NH}_4\text{OH}$ and from the incorporated radioactivity and amino acid analysis 1 mol of phosphate per mol of peptide was calculated; the total yield of phosphopeptide was 71 nmol . Successive Edman degradations of the phosphopeptide and electrophoresis of the products at $\text{pH } 6.5$ showed that the phosphate was present as shown in the sequence Gly-Arg-Gly-Leu-Ser(P)-Leu-Ser-Arg. This result was supported by studies with aminopeptidase and thermolysin. An identical labelling pattern was obtained with bovine brain protein kinase.

In these experiments Offord's formula⁹ was used to follow the change in electrophoretic mobility on the addition or removal of charged groups. We found that the average value for the change in charge on the addition of a phosphate group to a peptide was -1.35 at $\text{pH } 6.5$ in pyridine acetate buffer. When the phosphoserine was N-terminal, however, the phosphate group carried a more negative charge (approximately -1.6).

In kinetic experiments the cardiac kinase was used. Human myelin basic protein was used at the same molar concentration as the peptide to enable a comparison to be made. The effect of increasing ATP concentration on the production of phosphopeptide is shown in Fig. 1a. Michaelis-Menten kinetics were exhibited and from a double reciprocal plot a K_m value of $2.8 \times 10^{-5}\text{ M}$ was determined for ATP, similar to values reported for various protein kinases with proteins as substrates^{12,13}. The K_m found with myelin basic protein was $6.8 \times 10^{-6}\text{ M}$. At high concentrations of ATP, in excess of Mg^{2+} , there was a marked inhibition of the production of

phosphopeptide. A similar effect observed with phosphorylase kinase^{14,15} was attributed to the complexing of Mg^{2+} by ATP.

Half-maximal stimulation by cyclic AMP was produced at 10^{-7} M for both the synthetic peptide (Fig. 1b) and myelin basic protein as substrate. Values ranging from 0.4×10^{-7} to $3 \times 10^{-7}\text{ M}$ have been reported for a bovine cardiac protein kinase with histone (calf thymus type IIA) as substrate^{12,13}.

The effect of increasing concentration of synthetic peptide is shown in Fig. 1c. Again Michaelis-Menten kinetics were exhibited and from the linear double reciprocal plot a K_m of $2.4 \times 10^{-4}\text{ M}$ for the synthetic peptide with the cardiac enzyme was found. This value is similar to that reported for a peptic peptide substrate³. With myelin basic protein as substrate the K_m was lower ($5.3 \times 10^{-5}\text{ M}$). Miyamoto and Kakiuchi¹⁶ found a K_m of $2 \times 10^{-5}\text{ M}$ for the protein with a brain kinase. The V_{max} for the synthetic peptide was $1.47\text{ nmol } ^{32}\text{P}$ incorporated per 5 min and for the protein $0.36\text{ nmol } ^{32}\text{P}$ incorporated per 5 min . It should be emphasised that in the protein both threonine and serine sites are subject to phosphorylation⁵ (Table 1), whereas with the synthetic peptide only a single site was phosphorylated, thus the comparison of K_m and V_{max} is of limited value.

The effect of increasing time of incubation on the production of phosphopeptide in the absence and presence of cyclic AMP is shown in Fig. 1d. In 1 h very little phosphorylation of the peptide was observed in the absence of cyclic AMP. Thus it seems that the peptide is not able to dissociate the catalytic and regulatory subunits. A similar result was obtained with intact myelin basic protein whereas other studies¹⁶ have reported activation of protein kinase by both this protein and histone. But another sample of the same cardiac kinase as used above that had been frozen and thawed repeatedly showed less cyclic

AMP dependence and much greater activation with time when incubated without cyclic AMP (compare ref. 12).

The synthetic peptide was digested with proteolytic enzymes⁷ or subjected to Edman degradation⁷ and the products examined for substrate activity. Removal of the C-terminal arginine with carboxypeptidase B yielded a peptide, with relative mobility +0.38, which was readily converted to a phosphopeptide with relative mobility -0.06. From the N-terminal end glycine could be removed by Edman degradation without apparent effect on substrate activity, whereas the arginine was essential for activity. This requirement for arginine was confirmed by tryptic digestion, which also destroyed the capacity of the peptide to be phosphorylated. It could be argued that the loss of activity was simply due to the resultant shortening of the peptide but the need for arginine, rather than a neutral amino acid, is supported by the following observations. Although there are numerous serine and threonine residues within quite large tryptic peptides from myelin basic protein, only a few peptides in the tryptic digest were phosphorylated. All the peptides which were not phosphorylated had lysine or arginine to the C-terminal side of the serines whereas those that were phosphorylated contained arginine to the N-terminal side in trypsin resistant bonds⁶. One of these was the peptide Gly-N^G-monomethyl Arg-Gly-Leu-Ser-Leu-Ser-Arg. Preliminary evidence indicated that when the arginine was in the N^G,N^G-dimethyl form phosphorylation of the serine was prevented⁵. Moreover an examination of amino acid sequences around the site phosphorylated by cyclic AMP-dependent protein kinases in a number of non-globular and globular proteins (Table 1) shows that arginine is frequently found close to the serine that is phosphorylated.

Table 1 summarises the amino acid sequences in proteins phosphorylated by cyclic AMP-dependent protein kinases from a variety of tissues. Although in several cases the sequence Arg-X-Y-Ser-Z can be identified, there are also some instances where the arginine is only one residue removed from the serine or one further away. Hjelmquist *et al.*¹⁷ suggested a similar pattern and also that the serine should be surrounded by hydrophobic residues. The latter concept is not fully supported by the data in Table 1. It is puzzling that phosphorylase kinase phosphorylates the same type of site in phosphorylase b and in troponin I yet these sites are not those phosphorylated by the cyclic AMP-dependent protein kinases^{18,19}, which act on other serines in these proteins (Table 1).

Using probability factors listed by Chou and Fasman²⁰ for each amino acid at each position, 1-4, in a β bend, the probability of the sequences listed in Table 1 taking up a β -bend conformation was calculated. Although in several, a high probability emerged for the serine being in position 4 in a β bend, such high probability values were also obtained for sequences around serines in the basic protein which are known not to be phosphorylated.

Sites of phosphorylation in myelin basic protein were independent of the source of the kinase; purified kinases from rabbit skeletal muscle and bovine brain and cardiac muscle all produced the same labelling pattern (Table 1). In some substrates more than one site is phosphorylated at variable rates^{2,4,5,21}; however, no feature is apparent in the more readily phosphorylated sequences. This would suggest that once some minimum requirement is met, the rate of phosphorylation is governed by other factors.

Substrates in Table 1 have been categorised as non-globular and globular but no consistent differences are apparent in the sequences around the site of phosphorylation. Bylund and Krebs²¹ have shown that lysozyme, phosphorylase b and bovine serum albumin contain certain serine residues which are quite inactive as substrates in the native form, but which are readily phosphorylated when the proteins are denatured. This implies that in the native structure the sequence around these particular serines is constrained from taking up the conformation necessary for interaction with the active site of the enzyme. The sequence Arg-X-X-Ser is not a rare sequence

in proteins which do not act as substrates in their native form². Whether the failure of the kinase to phosphorylate these serines is due to the lack of accessibility or to the arginine being too remote spatially from the serine remains to be determined. Whatever the three-dimensional arrangement of the enzyme-substrate complex, however, we predict the need for a basic amino acid in close proximity to the serine to be phosphorylated. Chemical and physical studies with other synthetic peptides could help solve this problem.

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Tubulin requires an accessory protein for self assembly into microtubules

BORISY and Taylor have demonstrated a correlation between the presence of the colchicine-binding protein, tubulin, and the microtubular system of eukaryotic cells¹. Tubulin assembles *in vitro* into microtubules when a brain homogenate is warmed to 37 °C and is supplied with a cofactor, GTP (refs 2 and 3). These microtubules dissociate into tubulin subunits when the solution is chilled to 0 °C (refs 3 and 4), or Ca²⁺ is introduced^{2,4}. Tubulin can thus be purified by repeated cycles of assembly and disassembly^{4,5}. We have found, and others report similarly⁶⁻¹⁰, that some very high molecular weight polypeptides copurify with the tubulin in these conditions.

We have examined the role of these polypeptides in the assembly process and found that separation of the high molecular weight fraction from tubulin prevents self assembly of the tubulin; the colchicine-binding property of the tubulin is un-

affected. The high molecular weight fraction restores assembly competence when added back to the tubulin.

A preparation (for details, see Fig. 1) containing tubulin and the high molecular weight proteins was competent to reassemble into microtubules. We found two characteristic forms present in electron micrographs: 50-nm rings and 10-nm flexible fibres.

Tubulin has previously been reported to exist as dimers (molecular weight 110,000, $s_{20,w}$ 6S) and in an aggregate form of defined structure, a 49-nm ring complex ($s_{20,w}$ 36S)^{12,13}. Only 36S tubulin, but not 6S can assemble into microtubules^{12,13}. The 36S form is dissociated by high salt concentration (> 0.8 M NaCl) and reforms if the salt is dialysed away.

We have used this property to separate the constituents of the 36S particle. First, the microtubule pellet was dispersed in homogenising buffer containing 1 M NaCl at 0 °C. The treatment dissociated the 50-nm rings but not the 10-nm fibres, which were removed by centrifugation at 220,000g and 0 °C for 2 h. The supernatant, which was competent to assemble microtubules after the salt was removed, contained tubulin and only one of the high molecular weight polypeptides (Fig. 1d). The proteins were resolved by gel filtration, in the presence of 1 M NaCl on Sepharose 4B (Fig. 2a) or Sephadex G200 (similar, but not shown).

To remove NaCl the fractions were dialysed against medium containing 30 mM MES buffer; 0.2 mM MgCl₂; 1 mM EGTA;

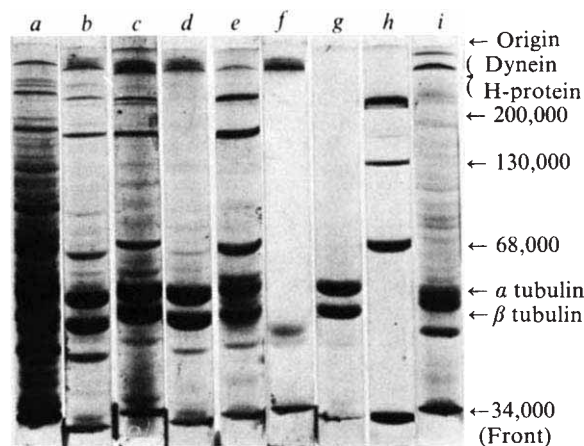


Fig. 1 Analysis of microtubule proteins by SDS-polyacrylamide gel electrophoresis. *a*, Whole brain supernatant; *b*, pellet after first assembly step; *c*, supernatant after three cycles of assembly-disassembly, without NaCl; *d*, supernatant after two cycles, 1 M NaCl in second disassembly; *e*, pellet from second cycle disassembly, 1 M NaCl; *f*, peak H from Sepharose 4B column; *g*, peak T from Sepharose 4B column; *h*, marker proteins: 34,000 aspartate transcarbamylase C subunit; 68,000 bovine serum albumin; 130,000 β -galactosidase; 200,000 myosin; *i*, flagellar proteins from *Naegleria gruberi*. Gel electrophoresis was carried out using the procedure of Laemmli¹¹, except that running gels contained only 6.7% acrylamide. Tubulin was prepared from rabbit brains following a method adapted from Shelanski⁵. Excised brains (about 9 g each) from adult rabbits were homogenised at 0 °C in a medium (12 ml per brain) containing 40 mM 2-(N-morpholino) ethanesulphonic acid, adjusted to pH 6.6 with NaOH (MES buffer); 0.3 mM MgCl₂; 1.5 mM ethyleneglycol-bis-(2-aminoethyl ether)N,N'-tetra-acetic acid (EGTA); 5 mM 2-mercaptoethanol and 1 mM GTP. The homogenate was centrifuged at 48,000g and 0 °C for 60 min. Glycerol was added (1 ml per 3 ml supernatant) and GTP was replenished (1 μ mol per ml supernatant) and the mixture was incubated at 37 °C for 30 min. Microtubules were collected by centrifugation at 100,000g and 25 °C for 60 min. The pellet was resuspended in homogenising buffer (1.5 ml/brain) at 0 °C to disassemble the microtubules, and was dispersed by agitation and expulsion through a syringe. After 30 min at 0 °C, the suspension was centrifuged at 120,000g and 0 °C for 40 min. Glycerol and GTP were added to the supernatant and microtubules were assembled by incubation at 37 °C as above. The second-stage microtubule pellet was resuspended in dissociation buffer (1 ml/brain) containing 40 mM MES buffer; 0.3 mM MgCl₂; 1.5 mM EGTA; 1.5 M NaCl; 5 mM 2-mercaptoethanol and 1 mM GTP. After 1 h at 0 °C, glycerol was added to a final concentration of 20% (v/v).

5 mM 2-mercaptoethanol and 4 M glycerol. GTP was added to the retentates to a concentration of 1 mM and the preparations were examined for microtubule assembly by electron microscopy (Fig. 3). Tested individually, peak H (from Fig. 2a), which contained the high molecular weight protein, and peak T, which contained tubulin, failed to form ring complexes at 0 °C and

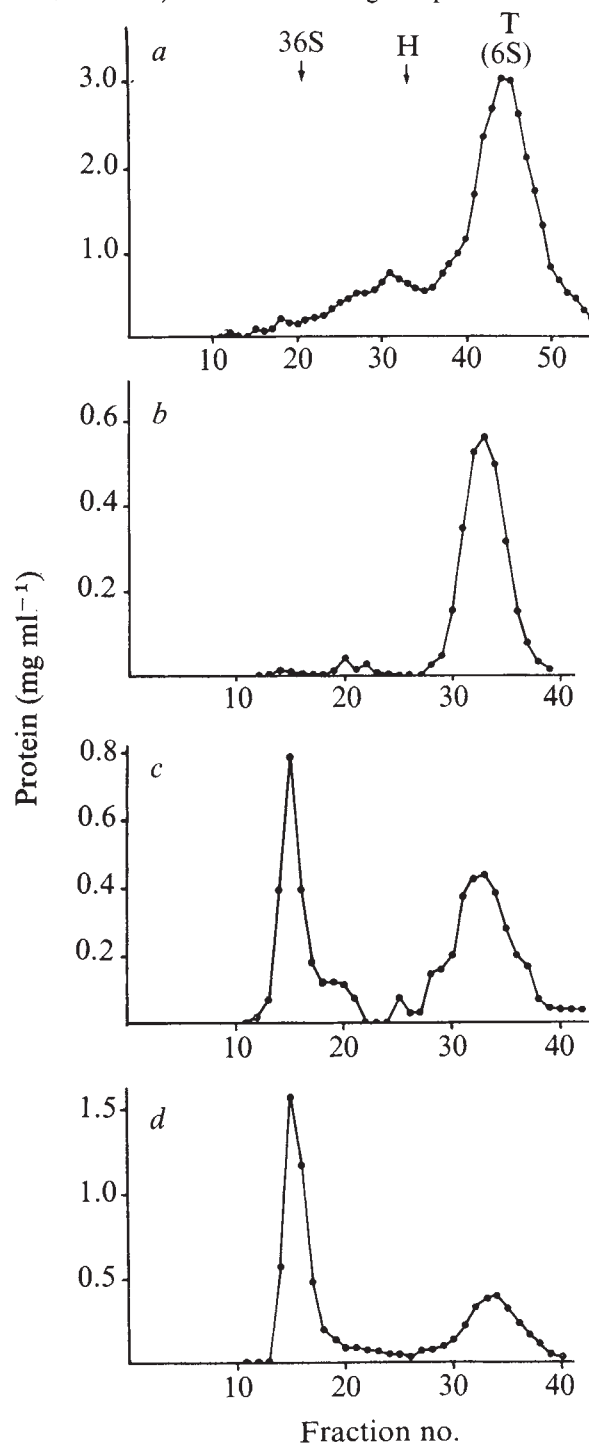


Fig. 2 Fractionation by gel filtration of microtubule disassembly products. *a*, Microtubules were disassembled at 0 °C, and products were dissociated by 1 M NaCl. Components were resolved by gel filtration in Sepharose 4B equilibrated in buffer containing 30 mM MES, pH 6.6; 1 mM EGTA; 0.2 mM MgCl₂; 1 M NaCl; 5 mM 2-mercaptoethanol and 20% (v/v) glycerol. Peak H contains high molecular weight protein and peak T corresponds to 6S tubulin; *b*, rechromatography of peak T after removal of NaCl, by gel filtration in Sepharose 4B, buffer as for *a*, but without NaCl; *c*, rechromatography of mixture of 1 mg H+5 mg T, preincubated with 1 mM GTP at 0 °C, column conditions as for *b*; *d*, microtubules disassembled at 0 °C, and products fractionated by gel filtration on Sepharose 4B without NaCl dissociation of 36S rings, buffer as for *b* and *c*.

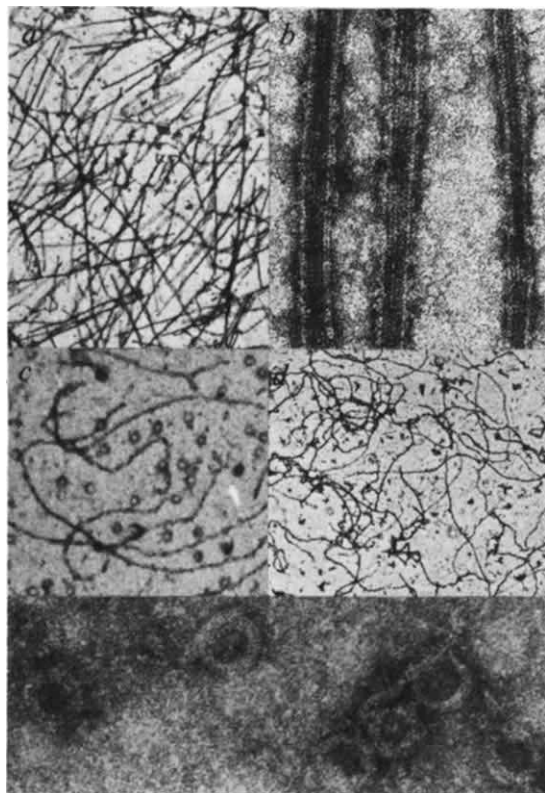
microtubules after incubation at 37 °C. Tubulin alone was passed again through Sepharose 4B, this time in the absence of NaCl, and eluted in the normal position for 6S dimer (Fig. 2b), indicating that 36S ring complexes had not formed. A mixture of tubulin and high molecular weight protein, allowed to reassociate in the cold in the absence of salt, formed the aggregate which eluted from Sepharose 4B in the position corresponding to 36S rings (Fig. 2c and d). Rings were observed in electron micrographs of the 36S peak (Fig. 3).

Mixtures of the two components incubated at 37 °C formed microtubules in large numbers ($> 10^3/0.003 \text{ mm}^2$ grid). In some cases, the 6S tubulin fraction prepared by Sepharose 4B chromatography formed limited numbers of microtubules ($< 30/0.003 \text{ mm}^2$ grid), but the high molecular weight protein was detected as a contaminant in these samples. Sephadex G200 generally gave a superior resolution of the components.

Early attempts to reconstitute an assembly-competent tubulin-accessory protein complex were unsuccessful until we included 2-mercaptoethanol in the buffers.

We estimate the molecular weight of the accessory protein to be about 360,000 from its mobility in SDS-polyacrylamide gel electrophoresis, by extrapolation from myosin (200,000), our largest available marker. The band matches the mobility of dynein isolated in flagellae from *Naegleria gruberi*. It seems likely that our microtubule accessory protein corresponds to the dynein-like proteins identified in brain tissue, which were also found associated with tubulin purified by the assembly-disassembly cycling procedure^{8,9}. These authors failed to find significant ATPase activity, characteristic of flagellar dynein, associated with their brain "dynein".

Fig. 3 Electron micrographs of tubulin preparations: *a*, mixture containing tubulin (2 mg ml^{-1}) and peak H protein (0.4 mg ml^{-1}) incubated for 30 min at 37 °C with 1 mM GTP ($\times 9,720$); *b*, same preparation at higher magnification, showing subunit structure of microtubules ($\times 184,200$); *c*, supernatant after microtubule disassembly in the third cycle of purification (see Fig 1c for protein composition) fibres lack any subunit structure similar to microtubules ($\times 40,090$); *d*, microtubule disassembly in 1 M NaCl, rings have disappeared but fibres remain ($\times 20,330$); *e*, supernatant after 220,000g centrifugation of NaCl-dissociated tubulin, dialysed and incubated at 0 °C with 1 mM GTP (protein composition shown in Fig. 1d) ($\times 214,800$). Preparations were negatively stained with 0.7% uranyl acetate, on carbon-coated Parlodion grids, with $25 \mu\text{g ml}^{-1}$ bacitracin as spreading agent.



The extreme size of the polypeptide causes difficulties in gel electrophoresis in many conventional systems and the protein penetrates poorly in gels of too high acrylamide concentration ($> 7\%$) or of too high bisacrylamide-acrylamide ratio. Either condition prevents formation of a distinct band in electrophoresis gels and would account for the failure of other workers to detect the accessory protein¹³.

We have evidence that the accessory protein is incorporated into the structure of microtubules rather than acting as an initiating factor or assembly catalyst. *In vitro* assembled microtubules band in sucrose density gradients at 1.289 g cm^{-3} (R.A.B.K. and R.H.H., unpublished) and both tubulin and the high molecular weight protein coincide with the band. Similarly, the accessory protein coelutes with the ring complex from a column of Sepharose 4B (Fig. 2d), although there is considerable difference in molecular size. When limiting amounts of accessory protein are added to pure tubulin, there is limited assembly into microtubules. These data are, however, based on electron microscopy which is only semi-quantitative and it has not been possible to determine an optimum stoichiometry for maximum reassembly of microtubules. The protein comprises about 15% by weight of intact microtubules (data derived from Fig. 2a), which corresponds to one 360,000 molecular weight protein per forty 55,000 molecular weight tubulin subunits, a ratio which suggests that each ring complex¹³ contains one accessory protein.

The results we describe here confirm the report by Weingarten *et al.* that a protein factor is required in addition to tubulin for microtubule assembly¹⁴. Weingarten found the assembly-promoting activity, tau factor, in a heterogeneous fraction containing many proteins which were not further resolved. Our high molecular weight protein has similar properties to the tau factor in that it permits assembly of 6S tubulin into microtubules at 37 °C and converts 6S to 36S tubulin at 0 °C.

Electron microscopy has failed to detect any large globular protein corresponding to the estimated size of the accessory microtubule protein, although tubulin monomers (55,000) can be distinguished in the structure of the microtubule. We speculate therefore that the accessory protein exists as a thin filament interconnecting the subunits, both in the complete microtubule and in the ring complex. Burns and Pollard⁸ have calculated the Stokes radius of their dynein-like protein from brain microtubules and conclude that it is highly asymmetric. One interesting possibility that emerges is the potential for different forms of accessory protein to modulate the properties of the resulting microtubule, thereby enabling identical tubulin subunits to form functionally distinct classes of microtubule. In this context, the amino acid sequence of tubulin has been found to be highly conserved in examples from widely divergent taxa¹⁵.

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'Single-stranded' DNA from ϕ X174 and M13 is cleaved by certain restriction endonucleases

DURING a systematic survey of the substrate specificities of a variety of restriction endonucleases, it was discovered that the *Haemophilus aegyptius* restriction endonuclease III (*Hae*III) specifically cleaved 'single-stranded' DNA from ϕ X174 and M13. Since these DNAs are not double-stranded¹ and restriction enzymes recognise duplex DNA with twofold sequence symmetry², this result was unexpected. This finding is significant with regard to the conformation of single-stranded viral DNA as well as the biochemical mechanism and physiological role of restriction enzymes.

Figure 1 shows a polyacrylamide gel electrophoretogram of the fragments produced by *Hae*III on ϕ X174 replicative form (RF), ϕ X174 (+) strand and M13 (+) strand DNA. For the duplex ϕ X174 RF DNA, nine bands are visible; the smallest fragment is not visible and the sixth band is a doublet thus totalling eleven fragments, as reported previously^{3,4}. Digestion of the 'single-stranded' ϕ X174 (+) DNA with *Hae*III produced eleven visible bands and similar digestion of M13 (+) DNA produced nine (Fig. 1). It was shown previously⁵ that M13 RF was cleaved by *Hae*III into ten fragments. The rate of cleavage of the 'single-stranded' DNAs was not as great as for the duplex RF; a 16 h digestion of the (+) strand DNAs was necessary to give a limit digest, whereas a 1 h period sufficed for the duplex RF. The reason for this difference in rates is unknown at present. Similar patterns were obtained when the *Streptococcus faecalis* restriction endonuclease I (gift of R. Wu) replaced *Hae*III. The cleavage site for both enzymes is GGCC.

Regions of duplex helical structure in the (+) strand DNAs (refs 6-8) seem to provide suitable sites for the specific cleavages. Heat-treated λ plac 5 DNA was not degraded, whereas the native double-stranded DNA was degraded to more than 50 specific fragments by *Hae*III in identical conditions. If, however, ϕ X174 (+) DNA was heated (98 °C for 5 min in 5 mM sodium phosphate (pH 7.4)-10 μ M EDTA) followed by quick chilling in ice before treatment with *Hae*III, it was degraded similarly to the untreated DNA. The ϕ X174 DNA seemed to reform appropriate duplex restriction sites readily whereas λ DNA did not. We cannot at present rule out the possibility that a single-stranded non-helical DNA is cut at a slow rate; however, the necessity of a duplex helical structure was shown also by studies with *Hpa*II (B. Baumstark, R. Roberts and U. RajBhandary, unpublished) and *Eco*RI (ref. 9) on appropriate synthetic substrates. Details of the mechanism of cleavage of 'single-stranded' DNAs are uncertain at present and are currently being investigated.

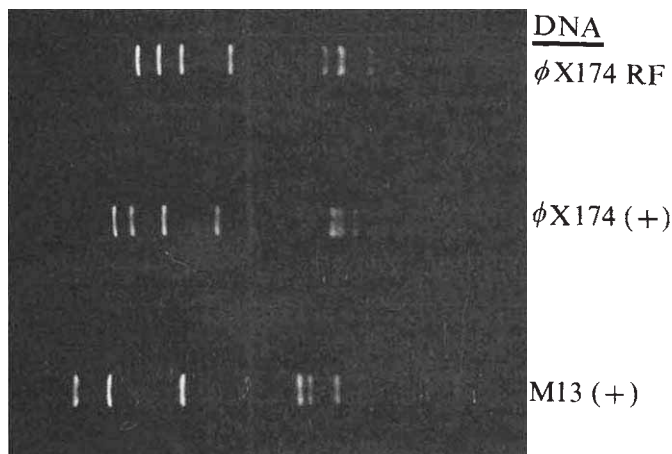


Fig. 1 Polyacrylamide gel electrophoresis of ϕ X174 RF, ϕ X174(+), and M13 (+) DNAs digested with *Hae*III. Both ϕ X174 DNAs contained *am3* and *ts41D* mutations (gifts of J. Dodgson and I. Nes); M13 (+) DNA was prepared as described previously¹⁰. The purification of *Hae*III was essentially as described by Roberts *et al.*¹¹ but in addition, the final enzyme stock solution was made 0.5 mg ml⁻¹ in bovine serum albumin. Each DNA sample (2 μ g) was incubated at 37 °C for 18 h in a 50 μ l reaction mixture containing 6 mM Tris-HCl (pH 7.9), 6 mM MgCl₂, 1 mM dithiothreitol and 6 U (ref. 11) of *Hae*III. Digestion was terminated by addition of EDTA to 50 mM. Each sample was made 15% (v/v) in glycerol and 0.03% (w/v) in bromophenol blue, then subjected to electrophoresis in 4% polyacrylamide cylindrical gels in Tris-borate EDTA buffer (90 mM Tris-borate (pH 8.3), 2.5 mM EDTA)¹² for 4 h at 200 V. The polyacrylamide gels (0.6 $\times$ 10 cm) were 3.8% (w/v) in acrylamide, 0.2% (w/v) in N,N'-methylene bisacrylamide (Bis), and were prepared in Tris-borate EDTA buffer-25% glycerol. After electrophoresis the gels were stained for 10 min with 10 μ g ml⁻¹ ethidium bromide and photographed during ultraviolet irradiation. Electrophoresis was from left to right; bromophenol blue migrated to 90% of the length of the gel.

*Hae*III specifically cuts the 'single-stranded' DNA into fragments of the same size as produced by cleavage of the RF. Figure 2 shows a formamide-polyacrylamide gel electrophoretogram of parallel *Hae*III digests of ϕ X174 duplex and 'single-stranded' DNA. The patterns on these denaturing gels are superposable except for the presence of two additional fragments (450 and 150 nucleotides in length; see arrows, Fig. 2) in the (+) strand pattern. It is thus assumed that both the RF and (+) strand are cleaved at the same GGCC sites, with the exception of the two additional fragments; the origin of these two fragments is unknown. The presence of the 450 and 150 nucleotide fragments was also observed as the fifth and eighth bands from the origin, respectively, in the non-denaturing gel (Fig. 1).

The following observations are consistent with the notion that *Hae*III, and not a contaminating enzyme, is specifically cutting the 'single-stranded' DNAs: 1, the similarity of fragment patterns for RF and (+) strand DNA (described here); 2, the

Table 1 Effect of restriction endonucleases on ϕ X174 and M13 DNAs

Enzyme	Recognition sequence	ϕ X174 RF	ϕ X174 (+)	M13 RF	M13 (+)
<i>Haemophilus aegyptius</i> (<i>Hae</i>) III	GGCC	11	11	10	9
<i>H. haemolyticus</i> (<i>Hha</i>) I	GCGC	≥ 14	≥ 14	≥ 12	≥ 9
<i>Streptococcus faecalis</i> (<i>Sfa</i>) I	GGCC	11	+	10	+
<i>H. influenzae</i> R _d (<i>Hind</i>) II	GTPyPuAC	13	0	1	0
<i>H. parainfluenzae</i> (<i>Hpa</i>) II	CCGG	8	0	13	0
<i>Arthrobacter luteus</i> (<i>Alu</i>) I	AGCT	> 10	0	> 10	0
<i>H. aegyptius</i> (<i>Hae</i>) II	Unknown	≥ 7	0	≥ 6	0
<i>H. influenzae</i> R _d (<i>Hind</i>) III	AAGCTT	0	0	0	0
<i>Escherichia coli</i> R (<i>Eco</i> R) I	GAATTC	0	0	0	0

The enzyme reaction conditions used were those previously reported for each nuclease using duplex DNA as substrate. The number of fragments produced by nuclease digestion of the RF DNAs was taken from refs 3-5 and our results. A + indicates that a number of specific fragments were observed, but the determination of their number was complicated by the presence of exonuclease in the *Sfa*I. The number of fragments generated by *Hpa*II on M13 RF is assumed to be the same as described⁸ for *Hpa*II.

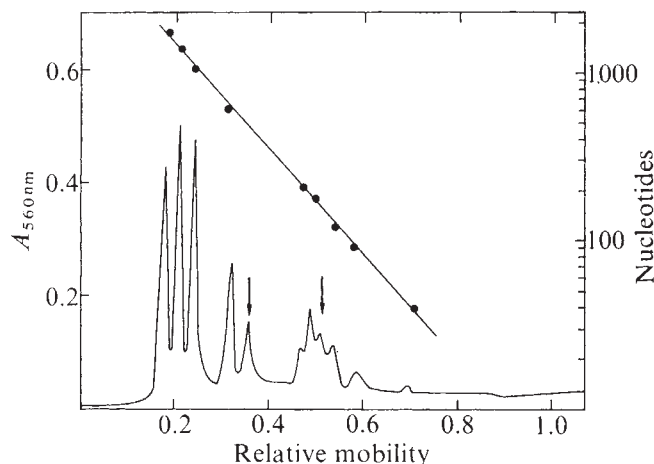


Fig. 2 Densitometric scan of *Hae*III fragments of ϕ X174 (+) DNA separated by formamide-polyacrylamide gel electrophoresis. ϕ X174 (+) DNA (3 μ g) was digested with *Hae*III as described in Fig. 1. Sodium acetate was added to the completed digest to 0.3 M, then 2.5 volumes cold ethanol was added. The DNA precipitate obtained after centrifugation was prepared for formamide-polyacrylamide gel electrophoresis as described by Maniatis *et al.*¹³. The DNA was dissolved in 25 μ l deionised formamide, heated at 100 °C for 2 min, then chilled in ice. Following the addition of 5 μ l of 25 mM sodium phosphate (pH 7.5), 75% glycerol and 0.2% bromophenol blue, the DNA sample was layered on a formamide-polyacrylamide cylindrical gel. The polyacrylamide gel (0.6 $\times$ 10 cm) was 4.25% in acrylamide, 0.75% in Bis, and was prepared in 98% deionised formamide with 20 mM sodium phosphate (pH 7.5). Electrophoresis was in 20 mM sodium phosphate (pH 7.5) for 1.75 h at 200 V. The gel was then stained overnight with 0.005% Stain's All in 50% aqueous formamide as described¹⁴. After destaining, the gel was scanned at 560 nm in a Gilford spectrophotometer equipped with a linear transport (—). In a parallel experiment, 2 μ g of ϕ X174 RF DNA was digested with *Hae*III and subjected to formamide-polyacrylamide gel electrophoresis. The peaks of stained RF DNA fragments from a densitometric scan were plotted against the logarithm of the length of the fragments in nucleotides: Z1 (1690), Z2 (1350), Z3 (1025), Z4 (600), Z5 (215), Z6 doublet (175), Z7 (120), Z8 (90), and Z9 (40) (●). The fragment lengths were taken from Middleton *et al.*³. Abscissa is the distance of the DNA fragments from the origin, relative to bromophenol blue. Arrows indicate the *Hae*III fragments of ϕ X174 (+) DNA which do not coelectrophorese with *Hae*III fragments of ϕ X174 RF DNA; no undigested DNA was observed at the origins.

enzyme activity that specifically cleaves RF and (+) strand copurifies throughout the enzyme isolation; 3, three separate *Hae*III preparations in this laboratory cleave both RF and (+) strand DNAs as well as one provided by C. Hutchison, University of North Carolina; 4, the ability to cleave both RF and (+) strand DNAs was lost simultaneously on either dilution or storage at -20 °C; and, 5, *Sfa* I (GGCC) gives products which are virtually identical.

The ability of nine restriction endonucleases to cleave ϕ X174 and M13 DNAs is summarised in Table 1. In addition to *Hae*III and *Sfa*I, *Hha*I (gift of W. S. Reznikoff) also cleaves the (+) strand and RF DNAs. It is not, however, a general capacity of restriction endonucleases to cleave 'single-stranded' DNA since *Hind*II, *Hpa*II, *Alu*I (gifts of I. Nes, M. Mann and H. O. Smith, and L. Maquat and W. S. Reznikoff, respectively) and *Hae*II cut RF DNAs but not the (+) strands. The probability of the 50% G and 50% C containing recognition sites of *Hae*III (GGCC), *Sfa*I (GGCC), *Hha*I (GCGC) and *Hpa*II (CCGG) appearing in any duplex region of the (+) strand should be equal. The failure of *Hpa*II to cleave (+) strand indicates some additional recognition site requirement.

*Hind*III and *Eco*RI (gifts of I. Nes and B. Weisblum, respectively) cut neither the RF nor (+) strand DNAs. Therefore, no new sites for these enzymes are present in the 'single-stranded' DNA which were absent in the RF.

These studies demonstrate that *Hae*III, *Sfa*I and *Hha*I specifically cleave (+) strand DNA apparently because of the

presence of a substantial amount of helical structure in the 'single-stranded' DNA. That ϕ X174 and M13 (+) DNAs are not devoid of ordered structure was indicated in previous studies⁶⁻⁸. Many types of folded structures are possible and all would provide identical fragments for any given enzyme, providing that all potential cleavage sites are in helical regions for each type. DNA structural considerations are not, however, the sole determinant of (+) strand cleavages; enzyme specificity also must be important since *Hae*III, *Sfa*I and *Hha*I digest (+) DNA whereas *Hpa*II gives no cleavages.

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DNA breakage caused by dimethyl mercury and its repair in a slime mould, *Physarum polycephalum*

A PRELIMINARY report from our laboratory showed that dimethyl mercury produced a radiomimetic breakage of slime mould DNA and suggested that the damage was repaired enzymatically¹. This communication further characterises the DNA damage and establishes that breakage is independent of DNA replication. It provides conclusive evidence for the existence of a dimethyl mercury repair system and shows that strains of *Physarum* differing in geographical origin have widely different sensitivity to dimethyl mercury damage.

The first set of experiments determined the S value and thus the molecular weight of *Physarum* DNA. Brewer's sucrose gradient techniques² were used, with nuclear isolation media of three different pH values. The observed S value of single-stranded DNA increased with increasing pH in the nuclear isolation medium. At pH 7.5, the calculated molecular weight of single-stranded DNA was comparable with Brewer's value² of 6.3×10^7 . At pH 8.0, 3.77×10^8 was obtained for single-stranded DNA, while at pH 9.0 it was 6.3×10^8 . S values were obtained using the modified Burgi, Hershy relationship³ and molecular weight calculations were based on Studier's⁴ relationship for DNA in alkaline sucrose solutions. As the molecular weight of DNA in each slime mould nucleus is 6.0×10^{11} (ref. 5), and the accepted chromosome number is 50 (ref. 6), the average weight of DNA per chromosome is 1.2×10^{10} . Therefore when nuclei are isolated at pH 9.0 the DNA is recovered as one molecule per chromosome. As the pH of the solution is decreased, the number of DNA molecules per chromosome increases. Bewer *et al.*⁷ suggested a subunit arrangement of single-stranded units in double-stranded molecules (2.3×10^8 daltons) isolated from slime mould nuclei at pH 7.5. Provided that the strain of slime mould used in our study was not fundamentally different in the organisation of DNA molecules in its

nucleus from the strain used earlier, our results suggest that the 'subunit' arrangement is produced by endonuclease which nicks the DNA at pH 7.5, while nuclear isolation at pH 9.0 allows no such nicking.

When *Physarum* was cultured in the presence of $\text{Hg}(\text{CH}_3)_2$ the S value of its DNA decreased considerably, giving a breakage pattern. In some cases it decreased from the normal 150 at pH 8.0, to approximately 10 at pH 8.0. This indicated that there were as many as 10^6 breaks per chromosome.

To find out whether breakage was independent of replication we labelled the DNA with ^3H -thymidine before the addition of the dimethyl mercury. Figure 1 shows that considerable breakage occurred even in the absence of DNA replication. In another series of experiments we found that damage caused by dimethyl mercury was repaired to some extent after removal of mercury by washing the cells once and resuspending them in fresh medium. After two washings followed by resuspension in fresh medium, repair was nearly complete within 24 h.

To obtain conclusive evidence for repair, we used bromodeoxyuridine (BrdU). A culture of *Physarum* was labelled with ^3H -thymidine in the presence of dimethyl mercury. After 24 h of incubation the cells were washed twice and resuspended in a medium containing unlabelled BrdU. (BrdU is incorporated in to DNA in patches where repair takes place, and was suitable for this experiment because it can be photolysed selectively by ultraviolet light (350 nm), allowing the DNA molecules to be broken where the original dimethyl mercury breakage and repair occurred.) When the nuclei of BrdU-treated plasmodia were isolated, part of the isolated DNA was exposed to ultraviolet light at 350 nm, while part of it served as a control. Figure 2 shows conclusive evidence that breakage caused by dimethyl mercury is repaired because the photolysed DNA molecules show the typical breakage pattern, while the molecules unexposed to high wavelength ultraviolet light show the unbroken repair pattern.

It is interesting that dimethyl mercury sensitivity varies from strain to strain of *Physarum*. The strain used in our experiments was a local Ontario strain of *P. polycephalum* while a

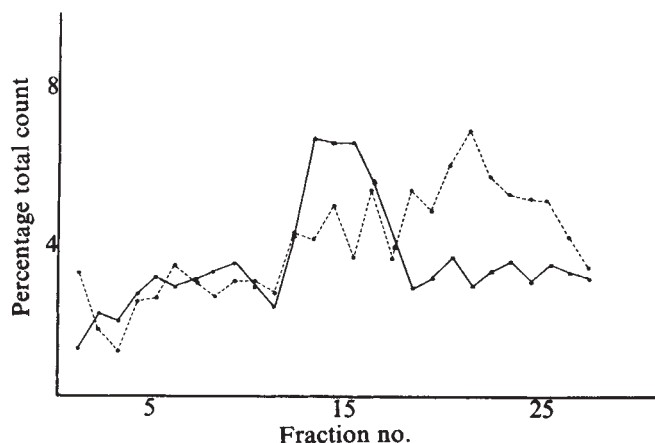


Fig. 1 Sedimentation of $\text{Hg}(\text{CH}_3)_2$ -treated *Physarum* DNA in an alkaline sucrose gradient. Cultures were treated for 24 h with 9.1 mCi ml^{-1} ^3H -thymidine (60 Ci mmol^{-1} methyl ^3H , New England Nuclear). With this treatment the labelled thymidine in medium is exhausted well before the end of the incubation period. At $t = 24 \text{ h}$ $\text{Hg}(\text{CH}_3)_2\text{OH}$ (0.60 mg ml^{-1}) was added to the medium and incubation continued for a further 24 h. Nuclei were then isolated using extraction buffer, pH 8.0. The nuclei were lysed in 0.5 M EDTA 1.0 N NaOH and 1% SDS. After lysis the nuclei for at least 20 min, the DNA was placed on a $5\text{--}15\%$ linear sucrose gradient and centrifuged for 1.5 h at $20,000 \text{ r.p.m.}$ in the SW25.2 rotor of a Spinco ultracentrifuge. Samples (2 ml) were collected from the bottom of the gradient. Albumin ($30 \mu\text{g}$) was added to each tube and the solutions were acidified and precipitated with trichloroacetic acid. The precipitate was collected on glass fibre filters, dried in glass scintillation vials and 5 ml of Omni-Scint (ICN Chemical and Radioisotopes) was added to each vial. Radioactivity was detected using a Beckman liquid scintillation detector. ●, $\text{Hg}(\text{CH}_3)_2$ -treated; ■, control.

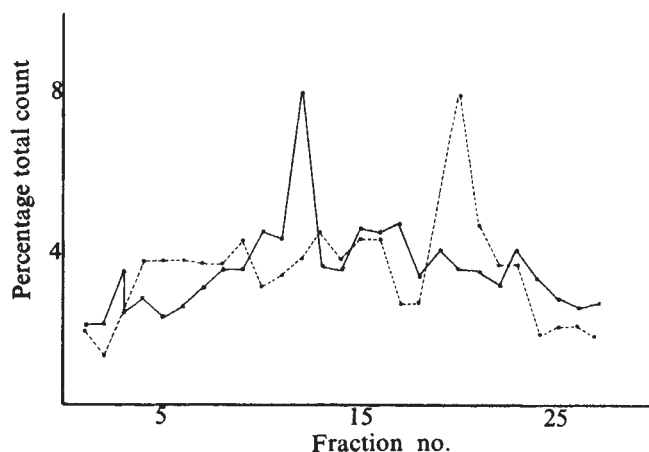


Fig. 2 Evidence for excision repair. Cultures were treated for 24 h in a medium containing ^3H -thymidine (2.5 mCi ml^{-1} and $\text{Hg}(\text{CH}_3)_2$. The cultures were washed twice (using low-speed centrifugation) and placed in medium containing BrdU ($20 \mu\text{g ml}^{-1}$), fluorodeoxyuridine ($5 \mu\text{g ml}^{-1}$) and Uridine ($20 \mu\text{g ml}^{-1}$). The cultures were then incubated for a further 24 h. Nuclei were isolated and lysed as described for Fig. 1. Samples (6 ml) of lysate were irradiated in open glass 10-cm Petri dishes for 30 min (350 nm , $26 \mu\text{W cm}^{-2} \times 100$) using a Gelman ultraviolet lamp. Irradiated lysates and unirradiated repaired lysates were analysed in the alkaline sucrose gradient as for Fig. 1. ●, Ultraviolet, (350 nm) irradiated; ■, non-irradiated.

strain of *Physarum* (M_3C) cultured originally at the McArdle Laboratory for Cancer Research in Madison, showed considerable resistance to the effects of dimethyl mercury. Even when the amount of dimethyl mercury present during treatment was double that used in the previous experiments, M_3C showed little or no DNA breakage. If comparable variations in sensitivity to dimethyl mercury-induced DNA damage exist in mammalian populations, the establishment of safe limits of dimethyl mercury in the environment is a more complex problem than might previously have been believed.

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Corrections in the catalogue of oligonucleotides produced by digestion of *Escherichia coli* 16S rRNA with T_1 RNase

SINCE its initial publication¹, the catalogue of oligonucleotides generated by T_1 RNase digestion of *Escherichia coli* 16S RNA has undergone a number of revisions and refinements^{2–5}. At present the literature contains two rather dissimilar documented versions of this catalogue^{2,3} and disagreements hold for 50% of sequences octamer and larger³. Confusion is not relieved by recent publications concerning the 16S rRNA primary structure^{6,7} that now incorporate some of the oligomer sequences of Uchida *et al.*³ but reject others, without resort to new evidence, however.

The situation demands definitive resolution. The uncertainties regarding oligomer sequences must be settled before one can use comparative approaches to the 16S rRNA sequence and before the ultimate goal of this research, the detailed molecular analysis of ribosome structure and its relationship to function,

can be approached. Therefore, we here review all ((T₁) oligomer sequences from the *E. coli* 16S rRNA catalogue for which there has been controversy or uncertainty.

All uncertainties in sequences are herein resolved (with the exception of two post-transcriptionally modified oligomers) due in part to the additional sequence information provided by a new endonucleolytic procedure (unpublished results of M. Sogin, D. S., L. Bonen and C. W.).

All relevant procedures—for generation of oligomers, their separation by two-dimensional electrophoresis, and for their sequence determination—are published in detail elsewhere³. Briefly, in determining individual oligomer sequences, we have used three types of (secondary and tertiary) endonuclease cleavages: (a) pancreatic (specificity for pyrimidines); (b) U2

(preferential cleavage of purines, cleavage of pyrimidines also under more stringent conditions³), and (c) the new activity, T3 (a complex, and so, useful specificity, outlined in Table 1). In almost every instance, a sufficient variety of fragments of an oligomer is generated by these methods to enable a sequence deduced from one subset of fragments to be confirmed by another.

Table 1 presents the correct sequences (and the incorrect versions they replace) for all the oligomers in question. Crucial secondary digestion products used to establish sequence in each case are shown, as are digestion products not found which, by their absence, disprove the incorrect versions.

It has been suggested that many of the differences between the two documented versions of the 16S rRNA catalogue reflect

Table 1 Sequence for the (T₁ nuclease) oligomers in the *E. coli* 16S rRNA catalogue

No.	A	B	C	D
7**	CCUAAACAACUG [C(UC)ACAACAUG] (22)	CCUAA, CA, CAUG, ACAACUG, UG	CCUAA, CAUG, ACAACUG	CAA
17*	AUAACUACUG [AUAACACUUG] (9)	UAA, CUA, CUG, CUACUG	CUA, AUAA, CUG	CUUG, CA
21***	CUAAUACCG [CUAAUACG] (25)	CUAA, CUA, UA, CCG, UACCG	CUAAUA, CCG	UACG
33***	AUUAG - 2 copies [AUUAG] (38)	UUA, G	AUU, AG	UA, UG
37**	CUCACCUAG [CUCACCUACAG] (31)	CUCA and CCUA, C(CU)AG	CUCA, CCU, AG, C(CU)AG, CUAG	CA, CAG (T3)
39**	AUCCCUAG [AU(C ₂ U)AG] (29b)	UCCCUA	AU, AG, CCCUAG	
43***	CCACACUG [CACACACUG] (61)	CA, CCA, CUG, CACUG		CCACUG
51***	ACUCCUACG (30b) [uncertain ³]	CG, CUCCUA, CUCCUACG	CG, CU, CCUA	
63*	UUAAUACUUUG [UCUAAUACUUUG] (1) [UUAAUACUUUG] ³	UUAA, UUA, UA, CCUUUG	UUA, AUA, CCUUUG, C(CUU)U, C(CU)U, CUU, UG, UUG	(UCU)A
71*	UACUUUCAG [UACUUUCUAG] (7)	UA, CUUUA	CAG, CUUU, UA	CUAG
75***	GCUG* [CCGCG] (111)			
89*	CUCAACCCUG [CUCAACCCUG] (30a)	CUCAA, CCUG	CU, C(CU)G, CUG	CCCUG
105***	CCCCCUG† [CCCCCUG] (93a) ³	CUG, C(CU)G, C(CCU)G	CUG, C ₂ UG, C ₃ UG, C ₄ UG, C ₅ UG, C ₆ U, C ₄ U, and so on	CCCCCUG, CCCCCUG
109***	AUACCCUG [AUACUCCG] (28)	UA, CCCUG	AUA, CUG, C(CU)G, C(CCU)G	CU, CCG
125	ACCCG† (106)			
127**	UUAAAACUAAAUG [UUAAAACUAAAUG] (5)	UUAAAA, etc. UG, CUAAAA, etc.		GUA, UCG
137***	UUUAAUUCG [UAAUCUUUG] (3)	UUUAA, UUCG	UUU, CG	UA, (CU ₄)G
143	ACAUCACG§ [ACCAUCACG] †	CA, UCCA, CG	CAU, ACAU, CCACG(?)	CCA, UCA
161**	CAACCCUUUACUUUG CAACCCUUUUU (AU, C ₂)G	CAA, CCCUUA, UCCUUUG	CCCUU, CCCUUUAU, CCUUUG	UG or (CCU)G, (C ₄ U ₅)A
165	AUAAACUG [AUUAAACG] (21)	UAAA, UAA, UA, CUG		UUA, CG
169**	UCAUCAUG [UCAUCAUG] (14)	UCA, UG, UCAUG	CAUG, UG	UCG
171**	CCCUUACG¶ [CCCCCUUACG] (37) [CCCCUUACG] ³	CCCUUA, CG	CG, CCCU, CCU, etc., CCCUU, CCUU, etc.	(C ₆ U ₂)A, CA
175***	CUACACACG C[(AC) ₃ , U]G (55b) [CA, CA, CUA]CG ²	CUA, CA, CG, CACG, CACACG		
179*	CAUACAAAG (50b)	UA, CA	CAU, CAUA, CAA	
181**	ACCUCG [ACUCCCG] (75b)	CCUCG	CCU, CG, ACCU	(C ₄ U)G
183**	ACCUCAUAAAG [ACCCUCAUAAAG] (19)	UA, UAA, CCUCA	CCU, ACCU, CAU, CAUA, AG	(C ₄ U)A

185***	CAACUCG C[AAC, U, C]G (65)	CAA, CUCG	CAA, CU, CG	
193***	AUCAG [AUCCAG] (70b)	UCA	CAG, AU	(UCC)A
203***	UACACACCG [U{(AC) ₃ , C ₂]G] (56b)	UA, CA, CCG, CACCG, CACACCG		
205***	CCCGG [†] [CCCCG] (101c)			
209**	AUUC AUG [AU AUUCG] (13)	UUCA, UG	AUU, CAUG, UG	UA, (UUC)G, CG
213**	CUUAAACCUUG [CUUAAACCUUG] (8)	CUUAA, CCUUG	CUUAAACCUU, CCUU, CG, CCUUG	UG
223**	AUACCCUCCUUA _{OH} ** [AUACCCU(CC, U)UA _{OH}] [‡]	A, UCA, (C ₄ U ₃)A _{OH}	CCU, CCUU, CCUUA _{OH} , AUCACCU	
	CUCG††	UCG	CU, CG	

Correct sequence for the (T₁ nuclease) oligomers in the *E. coli* 16S rRNA catalogue, the sequences of which had previously been disputed, ambiguous or uncertain. ³²P-labelled 16S rRNA isolated from 30S ribosomal subunits of *E. coli* B236 was digested with T₁ RNase and the digest fingerprinted by the two-dimensional electrophoretic procedure of Sanger and co-workers^{3,8}. The first (cellulose acetate) dimension was run in 0.003 M EDTA containing 7 M urea and brought to pH 3.5 with acetic acid; the second dimension (on DEAE cellulose) in 0.1 M pyridinium formate was brought to pH 2.3 with formic acid (unpublished results of M. Sogin, D. S., L. Bonen and C. W.). Individual oligomer spots, located by autoradiography, were sequenced by secondary and tertiary endonucleolytic procedures involving (1) pancreatic nuclease, (2) U2 nuclease, and (3) T3 nuclease activities³. The details of this last procedure will be published elsewhere, but in that it is novel, the procedure will be described briefly here. T3 activity is used chiefly in characterising pyrimidine stretches; it cleaves (T₁) oligomers most readily at U residues, and most slowly at C residues. Thus, for example, the six isomers of (C₂U₂)A would yield the following products, dominant products being overlined: (1) CCUUA → CCUU, CCU, CUU, CU, A; (2) CUCUA → CU, CUA, CUCU, (3) CUUCA → CUU, CA, CU; (4) UCCUA → U, CCU, CCUA, CUA, CU, A; (5) UCUCU → U, CU, CA, CUCA; (6) UUCCA → UU, CCA, U, CA. Each correct oligomer sequence (column A) is paired with the incorrect or uncertain versions thereof which have appeared in published versions of the 16S rRNA sequence^{5,6}. The former are assigned numbers designating their relative position in the 16S rRNA sequence^{6,9}; the latter, where appropriate, are given their number designations assigned by Fellner *et al.*². (*), (**), and (***) indicate respectively that an oligomer of that sequence is found in some other Enterobacteriaceae, most other Enterobacteriaceae, and in over 85% of prokaryotic 16S rRNAs screened^{9,10}. Secondary pancreatic nuclease digestion products, which are in all cases obvious, and concerning which no substantial disagreement exists, are not shown. The important secondary U2 and T3 nuclease digestion products in each case are shown in columns B and C respectively. Their sequences, where necessary, have been proven by further analysis. Column D lists digestion products predicted by the incorrect sequences, but not found. A dot placed over a nucleotide indicates that it has been post-transcriptionally modified. (Superscripts 3 and 7 indicate sequences reported in the corresponding references.)

*This sequence is tentative. Nevertheless, we feel that our data are inconsistent with the projected sequence of Fellner¹³ for these reasons: pancreatic nuclease products are a G, a C, and a spot containing two phosphates, that must be a dimer also by virtue of its electrophoretic mobility on DEAE cellulose in 0.1 M pyridinium acetate, pH 3.5 (PA) buffer; also the dimer must carry positive charge in that it runs faster than AC or CA, (but slower than the slowest monomer, G). T3 cleavage releases the same dimer (it is not cleaved further by pancreatic nuclease) and CG as the major products. For these reasons plus its position on the primary fingerprint³, we believe oligomer No. 75 to be a tetramer, of the form XCCG, where X could be the modified nucleotide previously identified¹³.

†We have detected C₄UG, C₂UG and so on and C₃U, C₄U and so on under conditions where C₄UG or C₄U, had they been present, would have been clearly recognised.

‡ACCCG does not exist in the *E. coli* strain we characterised fully³. But we cannot rule out the possibility that this represents a strain difference.

§ACAUCACG was not reported by us previously³. It appears to have been overlooked, as a minor contaminant in a cluster containing three other oligomers. This sequence has not been seen in other enterobacteria, but the closely related one, ACAUCACG, occurs in three of the five enterobacteria so far screened¹⁰.

¶We previously reported CCCCU... in this oligomer³, based not on definitive tertiary analysis, but position on the primary fingerprint. T3 digestion proves CCCU and CCCUU to be the largest possible pyrimidine stretches in the sequence.

||We are not certain of the nature, position, and number of the modifications here. We tentatively conclude a sequence CCCCG, but cannot rule out the alternative, that is two modified bases, proposed by Fellner¹³.

**In the catalogue of Uchida *et al.*³, the sequence of this 3'-terminal segment had been narrowed to the two possibilities shown. The sequence has also been determined by several other laboratories¹⁴⁻¹⁶.

††Fellner *et al.*² and Ehresmann *et al.*⁶ report (and place in sequence) four copies of CCUG but no copies of CUCG. Uchida *et al.* found two copies of CUCG and three copies of CCUG in the *E. coli* 16S rRNA catalogue³. We feel this does not represent strain difference. All five enterics screened contain at least one (most contain two) copies of CUCG and three or less copies of CCUG.

strain differences. This explanation we reject in all or almost all instances. Not only are all the oligomers hexamer and larger we report found in both B and K strains of *E. coli*³, but most of them are found in some (* in Table 1) or most (**) of the other Enterobacteriaceae screened, that is *Aerobacter*, *Yersinia*, *Proteus* and *Serratia*, and many of these in at least 85% of all other prokaryotes screened (***) as well⁹⁻¹².

It should be recognised that the oligomer sequence corrections made here do not bring into question the projected 16S rRNA sequence, unless the correction would change the composition of one of the pancreatic nuclease oligomers that overlap a given (T₁ nuclease) oligomer. At present no oligomers for which this latter condition holds are placed unequivocally in the 16S rRNA sequence⁶.

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Note added in proof: Yuki and Brimacombe have recently published¹⁷ evidence inconsistent with three sequences (Nos 143, 165, 175) above, but consistent with the alternative versions we reject. These discrepancies could all reflect errors in interpretation of certain mobilities in the pH 3.5

system, together in one case (No. 143) with 'overcutting' by ribonuclease U2. Specifically, confusion of UUA with CUG (No. 165), UA with CG (also No. 165) and in CUA with CCG (in No. 175) are possible. If this last explanation is correct, it necessitates the interchanging of the oligomers No. 175 (CUACACG) and No. 203 (UACACACG) in the 16S rRNA sequence.

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Radioiodine escape is an unexpected source of radioimmunoassay error and chronic low level environmental contamination

PROTEINS and peptides^{1,2} labelled with radioactive iodine (¹²⁵I or ¹³¹I) are commonly used in radioimmunoassays and other investigative procedures. Although decomposition of such labelled molecules can liberate iodide (I⁻) ions^{3,4}, which remain in solution, liberation of elemental radioactive iodine (I₂) has not been reported, as far as we know. Our findings reported here imply that, in conditions common in many radioimmunoassay laboratories, appreciable quantities of gaseous radioactive iodine can indeed escape from radioimmunoassay tubes.

While recounting several radioimmunoassays after a gamma-counter breakdown, we noted an unexpectedly rapid decline of radioactivity in the T tubes (capped 12 × 75 mm culture tubes containing only 100 µl of the ¹²⁵I-labelled protein antigen solution) which were being used to assess total radioactivity⁵. Because comparable decay was not seen in the other assay tubes, apparent "percentage-binding"⁴⁻⁶ seemed to increase progressively. The expected rate of isotopic decay (about 1% per day) was seen with tubes containing only inorganic ¹²⁵I solution, which seemed to rule out isotopic impurity as an explanation.

The possibility that the phenomenon was due to physical migration of isotope was investigated by serial gamma camera scintillography of two specially prepared T tubes. The gamma images (Fig. 1a–c) revealed progressive displacement of isotope from the solutions at the bottoms of the tubes to the tops. The central sections of the tubes remained devoid of detectable radioactivity. During this experiment, total radiation flux remained essentially constant, suggesting that the Parafilm caps had retained the iodine within the tubes, at least during the 2.5 h of observation. Of course, the material at the tops of the tubes would have been virtually undetectable during well-type gamma counting.

To determine whether the losses from the bottoms of such tubes would continue to equal the gains at the tops, several

ordinary T tubes which had already displayed count rate decays of 25% or more were rapidly frozen (to immobilise the solution) and cut in two. The bottoms and tops (inverted) were then counted separately. The count rate for each bottom was essentially the same as for the intact tube. The rates for the tops were significant (2×10^3 – 4×10^3 c.p.m.) but very much less than the missing radioactivity (about 3×10^4 c.p.m.). This finding, together with the apparent absence of radioactivity in the midsections of such tubes (Fig. 1c), implied that a major part of the translocated radioactivity was gaseous (presumably I₂) and had therefore escaped when the tubes were cut. That some of it had escaped even before the tubes were cut became evident when the shield tubes, in which these Parafilm-capped T tubes had been counted initially, also displayed significant radioactive contamination (again, not enough to account for the missing radioactivity).

These initial observations involved a very heavily iodinated preparation of labelled rat follicle stimulating hormone (FSH) (Table 1, line 1), but similar disappearance of radioactivity has been seen with much less strongly iodinated preparations of several pituitary hormones (Table 2). Table 1 contrasts the rapid apparent count rate decays for T tubes with the relatively slower decays displayed by tubes containing (1) various additives in solution; (2) precipitated trace-antibody complex (0-tubes), and (3) a sample of inorganic ¹²⁵I. With each of these labelled hormone preparations, the apparent rate of decay was minimal during storage at 4 °C in screw-cap jars but increased markedly when small volumes (100 µl) were placed in Parafilm-capped culture tubes and kept at room temperature. Accidental or deliberate puncturing of the caps further accelerated the apparent decay rate.

The trace preparations shown in Tables 1 and 2 were all iodinated essentially as described by Midgley⁵, using 0.125–0.75 mCi of ¹²⁵I per µg protein, 15–30 µg of chloramine-T, 60–175 µg of sodium metabisulphite and a reaction time of 40–128 s. About 150 mg of stable potassium iodide was used in the transfer solution. All had been purified by separating iodinated protein from fragments and free iodide, using gel columns (Biogel P-60) pretreated with either 5% ovalbumin or 0.2% gelatin. All had been immediately diluted to working strength in phosphate-buffered saline, pH 7.0, using 0.1% gelatin as the carrier protein.

The use of gelatin, which contains little tyrosine, as an alternative to 0.1% albumin (1% liquid egg white) had been adopted only recently⁷. Before second antibody precipitation, the assay tubes containing precipitates had held larger volumes of 0.1% gelatin solution than the T tubes (500 µl compared

Table 1 Apparent decay (AD) due to radioiodine escape from ¹²⁵I-labelled rat FSH in solution and as antigen-antibody precipitate (0 tubes) and effects of added egg white, gelatin, starch and sodium metabisulphite

Initial specific activity* (µCi µg ⁻¹)	Age of label, day 0	Additive, volume (µl)	c.p.m. (× 10 ⁻²)† and apparent % decay (%AD)					
			Day 0 c.p.m.	Day 2 c.p.m.	%AD	Day 7 c.p.m.	%AD	Day 11 c.p.m.
275	21 d	—	1,379	1,041	24.5	656	51.5	615
275	21 d	100% EW, 100	1,404	1,369	2.5	1,248	11.1	967
275	21 d	1% S, 100	1,401	1,153	17.7	718	48.5	606
275	21 d	0.1% G, 100	1,395	1,182	15.2	759	46.3	617
275	21 d	10% EW, 500	1,380	1,350	2.2	1,276	7.5	1,213
275	21 d	1% S, 500	1,390	1,343	3.4	1,235	11.2	1,140
275	21 d	0.1% G, 500	1,388	1,340	3.5	1,221	12.0	1,113
275	30 d	—	1,212	940	22.4	628	48.0	—
275	30 d	0.2mg M, 100	1,242	1,182	4.8	962	22.5	—
188	3 d	—	670	613	8.5	488	28.0	457
188	12 d	0 Tubes (n=6)	134	130	3.0	116	13.4	109
			±0.85	±0.97		±1.13		±0.39
Inorganic ¹²⁵ I			2,730	2,670	2.2	2,521	7.7	2,387

The distinctly overiodinated preparations (initially about 2.38 and 1.63 atoms ¹²⁵I per molecule, respectively) were prepared on February 28 and March 18, 1975 and, except for the 0 tubes and metabisulphite experiment, studied concurrently. Effects of adding EW, G and S were similar with both preparations. The rat FSH (rFSH), and the rat LH, TSH and prolactin (rLH, rTSH and rProl) shown in Table 2, were gifts from the NIAMD rat pituitary hormone distribution program. EW, egg white; G, gelatin; S, starch; M, metabisulphite.

*Expressed as µCi in protein peak per µg protein iodinated⁵.

†All counts are in hundreds, that is, 1,379 = 137,900 c.p.m. Each datum (except for 0 tubes) is the mean of duplicate tubes.

‡Note complete prevention of ¹²⁵I escape during 11 d at room temperature.

Table 2 Apparent radioactivity decay in ^{125}I -labelled pituitary hormones of high and low initial specific activities

Labelled hormone	Initial specific activity* ($\mu\text{Ci } \mu\text{g}^{-1}$)	Age of label day 0	c.p.m. ($\times 10^{-3}$)† and apparent % decay (%AD)				
			Day 0 c.p.m.	Day 4 c.p.m.	%AD	Day 7 c.p.m.	%AD
rFSH	63	< 2 h	846	765	9.6	710	16.1
rLH	400	17 d	939	650	30.8	530	43.6
rLH	62	< 2 h	677	609	10.0	577	14.8
oLH	230	15 d	1,120	996	11.1	906	19.1
rTSH	279	15 d	1,194	908	24.0	771	35.4
rTSH	80	< 2 h	984	862	12.4	798	18.9
rProl	85	2 h	894	792	11.4	753	15.8
Inorganic ^{125}I			2,730	2,670	2.2	2,521	7.7

See legend of Table 1 for identities of rat hormones. The preparation oLH is LER-1056 C2, a highly purified ovine luteinising hormone given by Dr L. Reichert.

*See first footnote, Table 1.

†See second footnote, Table 1.

with 100 μl) as well as other proteins (immune and non-immune sera) not present in the T tubes. Table 1 shows that the presence of protein solution (even gelatin, in adequate volume) can retard, or even totally prevent, radioiodine escape.

A complete explanation of this phenomenon is beyond the scope of this report. There are two possibilities. One is that iodine can be released from covalent bonding to tyrosine residues as a result of radiation damage³. The other is that it is released from non-covalent radioiodine-protein bonds. It is known (Fig. 8b and c of Yalow and Berson³) that free radioiodide does appear on storage of ^{125}I -labelled protein preparations. This radioiodide may be converted, at least in part, to radioiodine. The facts that (1) escape of radioiodine accelerates when trace solutions are brought to room temperature and exposed to air, and (2) addition of metabisulphite can partially prevent this escape (Table 1) suggest that some type of chemical oxidation is involved.

Escape of radioiodine poses two sets of problems. The most immediate is the potential hazard to laboratory personnel who may be undergoing chronic, low level exposure to an environment contaminated with radioiodine. The extent of this hazard would be expected to vary widely, because of variations in rates of trace protein decomposition (Tables 1 and 2) and amounts of radioiodine-labelled protein subjected simultaneously to destabilising conditions. Thyroid scans of the four individuals who would have received the greatest exposure during the 2-month period throughout which ^{125}I had been escaping in our laboratory (that is before the institution of the precautions discussed below) revealed no detectable

neck radioactivity (less than 0.05 μCi). Conditions during this short period (moderate numbers of assay tubes, high rate of air exchange) did not favour maximum environmental contamination. We have, however, calculated that laboratory conditions could exist in which air concentrations of radioiodine escaping from assay tubes could exceed US Nuclear Regulatory Commission air concentration limits (10 CFR 20, Appendix B) by as much as 500 times. (To reach these extreme limits, large numbers of assay tubes would have to be kept at room temperature in a small space with a poor rate of air exchange. Nevertheless, since this report was prepared, we have learned of one laboratory in which detectable neck radioactivity was encountered in a technician who worked in a room in which discarded assay tubes were stored.)

Radioiodine escape, at any rate, is clearly undesirable⁸, particularly with ^{125}I (half life 60 d). Allowing it to occur unchecked now seems inexcusable, since it can be minimised by the following simple precautions: adding a tyrosine-rich carrier protein (such as albumin) to the solution in which trace is stored; keeping stored solutions tightly capped and cold; adding a sufficient volume of albumin solution to all assay tubes. In the important case of the predominant assay tubes, those containing antigen-antibody precipitate, the small but distinct decay due to iodine escape during storage at room temperature (0-tubes in Table 1) can be blocked for at least 21 d by covering the precipitates with 500 μl of 1% albumin solution immediately after decanting the supernatant. (This innovation has noticeably improved assay precision in our laboratory). These precautions, however, would not be expected to prevent protein-radioiodine dissociation, but only to conceal its development by trapping the isotope in solution, as ^{125}I -labelled albumin. Progressive distortion of measurements of antigen-antibody interaction^{4,6} would still result. Nonspecific labelling and/or label lability could also complicate tissue localisation studies⁹.

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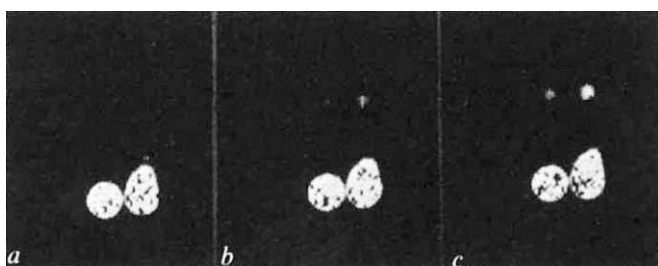


Fig. 1 Successive gamma camera images of two Parafilm-sealed (12 $\times$ 75 mm) culture tubes containing ^{125}I -labelled rat FSH solution, showing progressive saltatory displacement of ^{125}I from solution. Times: (a) 15-35; (b) 75-95; (c) 135-155 min after preparation. The gamma camera was a Searle Pho-Gamma HP coupled to an Intertechnique Cine 200 Data Collection System. Each tube contained 0.1 ml of a concentrated solution of the ^{125}I -labelled FSH shown on line 1, Table 1, and was sealed with three or four layers of Parafilm. About 15 min after preparation, the tubes were mounted upright in the field of the (pinhole-columnated) gamma camera. Although they were not moved during the series of 20 min exposures, the tube on the right had been shaken manually (before mounting) to simulate travel in an automatic gamma counter. This seemed to accelerate subsequent volatilisation of radioactivity. The use of concentrated solution was dictated by gamma camera availability. Similar pictures of ordinary T tubes would have required longer exposure periods.

Erratum

In the article "Methyl group as a probe of chirality in Raman optical activity" (*Nature*, **255**, 458; 1975) the author's name should be L. D. Barron and not as printed. Parts of the figures in the original article could not be seen clearly so they are reprinted below on a larger scale.

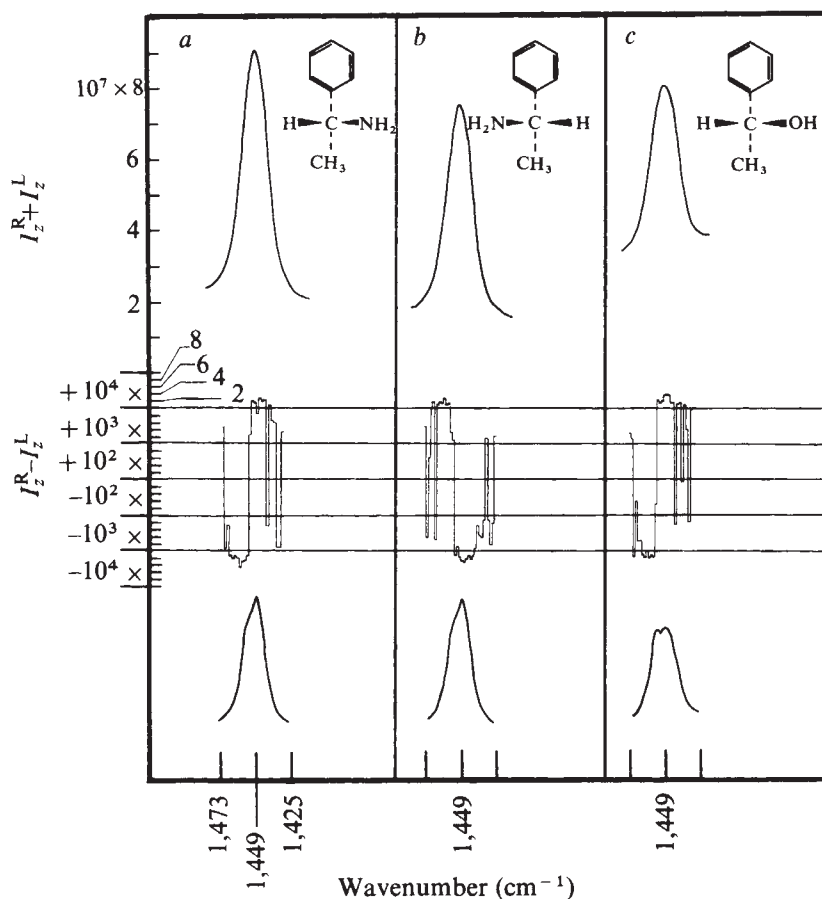


Fig. 1 The depolarised Raman circular intensity sum (top) and difference (centre) spectra, as recorded, of methyl asymmetric deformations in: *a*, (+)- α -phenylethylamine; *b*, (-)- α -phenylethylamine; *c*, (+)- α -phenylethanol. At the bottom is a sum spectrum at high resolution. Instrumental conditions: laser wavelength 4,880 Å, laser power 1 W, slit width 1,500 μ m for the top and centre spectra and 200 μ m for the bottom spectra. The absolute configurations are taken from ref. 10.

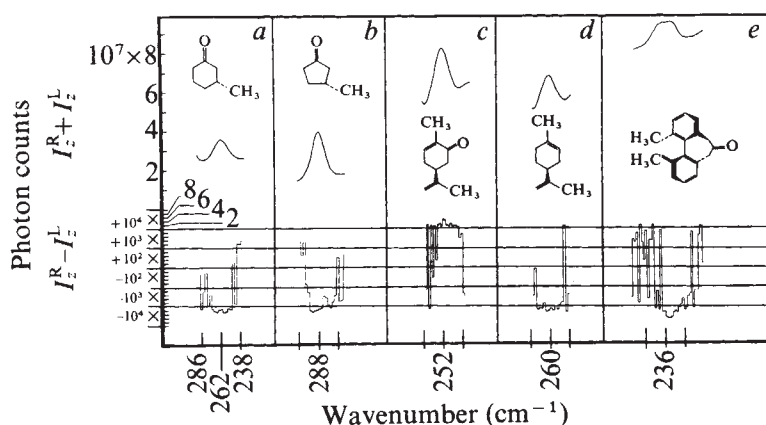


Fig. 2 The depolarised Raman circular intensity sum and difference spectra of methyl torsions in: *a*, (+)-3-methylcyclohexanone; *b*, (+)-3-methylcyclopentanone; *c*, (+)-carvone; *d*, (-)-limonene; *e*, a saturated solution of (+)-dimethyldibenz-1,3-cycloheptadiene-6-one in acetone. Instrumental conditions as for the top two spectra in Fig. 1, except that a laser wavelength of 5,145 Å was used in *e* to minimise fluorescence.

reviews

THIS volume* contains 18 papers presented in 1972 at a conference attended by scientists, historians and philosophers at the Villa Serbelloni in Bellagio, Italy. Each paper is followed by comments of one or more of the participants, usually Bernard Rensch. If the subject of this conference was, as Ledyard Stebbins (p. 285) claims, reduction in biology, then few of the participants addressed themselves to the issue.

The two boundaries between natural phenomena which are of greatest interest in arguments over reduction are that between the mind and physiological processes and that between biological processes in general and the subject matter of physics and chemistry. Until quite recently, all of the carefully worked out examples of reduction have concerned reduction within physics, the reduction of one physical theory to another. Thus, the next step would seem to be the analysis of similar examples within biology. Furthermore, these are exactly the examples that professional biologists are best prepared to deal with constructively. But the topic never arose. Instead, most of the papers which touched on reduction at all, dealt with the reduction of mind to physiological processes and a few concerned the reduction of biological processes to physics and chemistry.

The papers divide fairly naturally into three groups: historical, biological, and philosophical. The philosophical papers divide somewhat less easily into those which are straightforward philosophy and those which are 'philosophical' in the pejorative sense. The papers by Ernest Boesinger, June Goodfield, and G. Montalenti are primarily historical. Boesinger traces the fate of the evolutionary hypothesis after Lamarck and Darwin, giving special attention to the bizarre situation which still prevails in France. Goodfield attempts to derive some conclusions about reductive strategies by studying the methods and results of a variety of biologists in the 19th and 20th centuries. Montalenti compares mechanistic reductionism and holistic vitalism in Democritus, Aristotle, and Darwin. Gerald Edelman's paper is also historical to some extent, discussing as he does the recent history of the theory of clonal

Philosophy of reductionism in biology

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Aristotle—mechanistic reductionism and holistic vitalism. Taken from Hartmann Schedel Liber Chronicarum (Nuremberg Chronicle), Nuremberg, 1493.

Roman Picture Library

selection in antibody formation. The papers by Dobzhansky, Stebbins and John C. Eccles are mainly biological. If there was ever an excuse for the ill-informed parodies that so often pass for discussions of the synthetic theory of evolution, Dobzhansky's lucid synopsis eliminates it once and for all.

The papers by Morton Beckner, Dudley Shapere, Francisco Ayala, Donald Campbell, and Karl Popper are truly philosophical. Beckner exploits the distinction between hierarchically arranged theories and hierarchically organised natural systems. Shapere expands upon the difference between compositional and evolutionary theories and the different sorts of problems which give rise to them. Donald Campbell sets out his views on evolutionary epistemology, views shared in part by Popper and Henryk Skolimowski. Just as biological species adapt to their changing environments by a process of variation and selective retention, individual organisms come to learn about their environments and successive scientific theories come to characterise the real world with ever increasing accuracy. One problem with this

philosophical thesis concerns the appropriate adjective required to modify the term 'variation'. In what sense are mutations 'chance'? In what sense is learning and the development of science 'blind'?

Ayala presents a surprisingly informative and sensitive linguistic analysis of "evolutionary progress", surprising because such analyses are rarely informative and even more rarely produced by practicing scientists. Stebbins also deals with the notion of progress in his contribution. Peter Medawar presents an uncontroversial comparison between levels of generality in geometry and the natural sciences. Skolimowski, on the other hand, presents an unnecessarily petulant attack on the conventional notion of rationality in science. The tone of Skolimowski's paper is especially unfortunate because the issues are both important and inherently liable to distortion and caricature.

Large chunks of the papers by Rensch, Eccles, W. H. Thorpe, and Charles Birch exemplify what can happen when a noted scientist tries his hand at 'philosophising': these papers are uncomfortable reading. The biology is accurate and often inherently fascinating—for example, Eccles' discussion of split brain experiments and Thorpe's description of migration and exploratory learning—but too often their attempts to set out philosophical theses are as embarrassing as the efforts of an ageing *diva* trying to sing the latest pop song. Although Shapere values interdisciplinary endeavours, he replies to these poorly formulated views in the only way a professional philosopher can (pp. 256, 258). Just as scientists are entitled to establish standards of competence for their undertakings, philosophers have a right to expect at least minimal competence in theirs.

I have, however, saved the best part of the book until last. Monod did not present a formal paper at the conference; instead, he defended his book *Chance and Necessity*. The ensuing discussion points up, in the most direct manner possible, both the obscurity and importance of the problems which surround the issue of reduction. Several of the papers in this volume go a little way towards reducing the obscurity, and the emotional exchange between Monod and Skolimowski attests to the importance of these issues. I could not recommend that anyone read this volume cover to cover, but a few of the papers are worthwhile and the final 20 pages, in which the participants quiz Monod, should not be missed.

David L. Hull

**Studies in the Philosophy of Biology: Reduction and Related Problems*. Edited by Francisco J. Ayala and Theodosius Dobzhansky. Pp xix+390. (Macmillan: London and Basingstoke, September 1974.) £12.00.

THIS volume* is the result of a gathering in April 1974 of scientists from different disciplines with a common interest in the thymus. Fifty-three contributions on various aspects of the thymus unfortunately make heavy reading and because they are all formal papers it is difficult to measure the success of the meeting in providing a forum for the exchange of ideas. Rarely does one find proceedings that do justice to the tone and atmosphere of a meeting.

Although all immunologists have believed in the importance of the thymus, most in the past have paid only lip-service to the notion that thymocytes and T cells exert their influence through soluble factors. In the last four years, however, this has changed and now everybody is chasing factors. The inevitable result has been an explosion in the literature and a proliferation of factors which reflects more the individuality of the scientists than the molecular nature of the factors themselves. To a certain extent this book provides an opportunity to compare the many different approaches of the workers in the field, but this could have been made easier by a more extensive editorial summary.

The volume is divided into six sections, by far the most readable of which are the first (the T cell story) and the last (perspectives of the role of thymus factors in immunity), which can be recommended to any reader. The filling of this sandwich comprises sections on soluble factors and T cell development, the preparation of thymic factors, their activity *in vitro* and their activity *in vivo*. The articles are generally well presented and illustrated but many of course have been superseded in the year it has taken to publish this work. This is a universal fault with conference proceedings and it is a shame that it has happened in this case because this is a very useful reference work. Although it contains much on thymic hormones, it has little to say about the functioning of immune responsiveness genes—which is a pity as this is a fascinating and rapidly expanding area.

What the volume does achieve is to highlight the lack of standardisation of thymic factors. One may hope that an attempt to achieve agreement in this area will arise out of meetings such as this. Another salutary achievement is

Role of the thymus in immunobiology

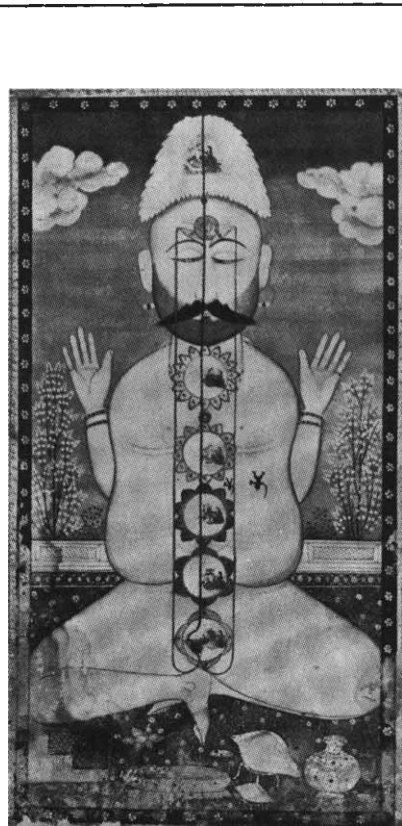


Diagram of the six cakras in the subtle body, Kangra, Himachel Pradesh. Gouache on paper, eighteenth century. "The first and fundamental image which reflects inner facts is the 'subtle body', which is shared by all Indian traditions, as well as by Indian medicine. It is experienced as that immense ramification of channels of energy which flows through the entire body." Taken from *The Body as a Medium of Expression* edited by Jonathan Benthall and Ted Polhemus. Pp. viii + 333. (Allen Lane, Penguin Books: London, May 1975.) Hardcovers £7.00; paper £3.50.

in the underlying current of caution expressed in interpreting cellular function by the presence or absence of differentiation antigens on cells exposed to thymus factors.

Norman A. Staines

SINCE the discovery in 1968 of genetic athymia in 'nude' (*nunu*) mice, the continuing study of these animals is throwing new light on the functions of the mammalian thymus. About 200 papers have been published so far, and an analysis of the present state of the subject would obviously be very useful.

Dr Rygaard's book† does not satisfy this need adequately. It was, in fact, published in Denmark in 1973 and is now being marketed more widely, without change or addition. Unavoidably, in a fast moving field it is already out of date. Suffice it to say that the majority of research reports on the nude mouse belong to the period after the book was written. Furthermore, out of 83 papers known to me from the period up until the end of 1972, 34 are not listed in the references.

A quarter of the book is devoted to the author's own line of experimentation—demonstrating that the athymic animal accepts grafts from a variety of vertebrate species. Data of body and organ weights of nude and control mice are presented in convenient tables. Rather unexpectedly, spleen and lymph node weights were usually higher in nude mice than in normal controls. The levels of various serum proteins, including gamma globulins, were also normal. The problems raised by some of these data are pointed out but not followed up.

The coverage of other topics is rather uneven and cursory. No tables, graphs or illustrations from other workers' original reports are incorporated. Work on the production of humoral antibodies is summarised rather too briefly.

The last two chapters are based on the author's own finding that no spontaneous tumours developed in any of over 11,000 nude mice recorded. He rightly points out that the short life span of these animals permitted an average observation time of only 4 months, but proceeds to deemphasise this difficulty on grounds that are not altogether decisive. The statement, for example, that auto-antibody production is rare in nudes can be questioned. Essentially, on these bases, the author expresses doubts about the theory of immunological surveillance and declares his conversion to the ranks of agnostics in this respect.

The book is written in a lively style and is attractively produced. In spite of its limitations, it is likely to stimulate interest in the study, through the nude mouse mutation, of the thymus. E. M. Pantelouris

**Thymus Factors in Immunity*. (Annals of the New York Academy of Science, Vol. 249.) Edited by Herman Friedman. Pp. 547. (New York Academy of Sciences, February 1975.) \$39.00.

Corrigendum. In the review of *Registry of Mass Spectral Data: Volumes 1-4* (*Nature*, 257, September 4, 75, 1975), the collective price of the four volumes was incorrectly stated as £250.00. The price should have read £155.25.

†*Thymus and Self: Immunobiology of the Mouse Mutant Nude*. By Jørgen Rygaard. Pp. 193. (Wiley-Interscience: London and New York, March 1975.) £6.50.

Mechanics of Polymers. By R. G. C. Arridge. Pp. ix+246. (Clarendon: Oxford; Oxford University: London, June 1975.) £6.50.

Books on the mechanical properties of polymers range between two extremes: the practical approach is to present large quantities of data held together with theoretical interludes, whereas the pure approach is to stick mainly to theory and only introduce data occasionally.

This book is predominantly of the second sort. There is a thorough discussion of elasticity theory, including large strain elasticity and anisotropy, and a chapter on time-dependent elasticity theory, but these are left hanging as the chapter on applications to polymers discusses mainly transition temperatures and test methods.

The treatments of anisotropy and of yield and fracture are more integrated, although there are relatively few examples. The discussion of fracture is very short.

This book is too uneven to be a good general text, although individual chapters may prove valuable. Most of the material is also in Ward's *Mechanical Properties of Solid Polymers* (1971). An understanding of vectors and tensors is required. Most of the time SI units are used. Some of the diagrams, particularly of chemical structures, are unclear.

P. D. Calvert

Applications of High-speed Liquid Chromatography. By J. N. Done, J. H. Knox and J. Loheac. Pp. vii+238. (Wiley-Interscience: London and New York, January 1975.) £6.50.

THE technique of high speed, high pressure, liquid chromatography (HPLC) is one of the more rapidly expanding areas of analytical chemistry: it now rivals gas chromatography in terms of practical importance. The primary advantage of the method is that it can separate compounds which are not readily amenable to gas chromatography; for example, polymers, high molecular weight compounds, and ionic and strongly polar species such as steroids and sugars, and so on.

In the modern analytical laboratory any decision to invest in a new technique is often dependent on an assurance that it can solve a specific problem. This book aims to assist in this decision and also to provide a much needed practical guide to the field.

After a brief but effective coverage of the technique and theory of high speed liquid chromatography the major section of the book consists of examples of selected chromatograms taken from the recent literature. The 150 examples

shown illustrate the wide range of compound types amenable to HPLC and cover hydrocarbons and petroleum, acids, amines and nitrogen compounds, phenols and antioxidants, sulphonic acids and dyestuffs, insecticides and herbicides, nucleotides and nucleic acid bases, pharmaceuticals, steroids and natural products. Each chromatogram includes details of column material, column length, packing and particle size, operating temperature, pressure type of detection and sensitivity.

As the authors correctly point out, the rapid advances in this field will lead to improvement in some of the chromatograms but the operating conditions will not undergo any radical changes.

Altogether this is an excellent book, concise and well documented and providing the essential information for anyone contemplating the use of this important technique.

B. Fleet

Books brief

Compaction of Coarse-grained Sediments, (Developments in Sedimentology 18A.) Edited by George V. Chilingarian and Karl H. Wolf. Pp. 552. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl. 130; \$54.25.

THIS latest contribution to the series *Developments in Sedimentology* is the first of a two-volume appraisal of compaction of coarse-grained sediments, and complements an earlier volume dealing similarly with argillaceous sediments. For such a highly specialised subject, embracing so many disciplines, the editors have succeeded in assembling a balanced collection of well presented and illustrated papers which should largely satisfy geologists and engineers working in this field. Most of the chapters are essentially of a review nature and contain comprehensive bibliographies; as such, the book should serve as an invaluable source of reference.

Of specific interest to geomechanics are chapters dealing with experimentation and mechanics of sand compaction (Allen and Chilingarian), its effects on such properties as sediment porosity, permeability, strength and so on (Ingles and Grant), and a detailed presentation of mathematical models illustrating problems arising from the compaction of porous solids (Raghavan and Miller).

Sedimentologists are catered for in papers dealing with compaction of carbonate sands (Coogan and Manus), a case study demonstrating how

differential sand-mud compaction can determine the geometry and distribution of elongate sandstone bodies (Brown) and the use of geophysical well logs in recognising depositional environments and sediment compaction (Allen). Bissell and Chilingarian review the magnitude and possible causes of hypersubside in various sedimentary basins in North America as well as reporting on localised subsidence related to oil and water extraction.

The second part is eagerly awaited.

Brian Waugh

Chemical Oceanography. Edited by J. P. Riley and G. Skirrow. Volume 1. Pp. xx+606. £18.50; \$49.00. Volume 2. Pp. xx+647. £19.50; \$51.50. (Academic: London and New York, June 1975.)

THIS edition up-dates the previous one of about a decade ago and these volumes benefit from more quantitative descriptions of marine chemical phenomena. Refreshingly, an attempt has been made to standardise units of measurement throughout these volumes. Most of the chapters contain a complete range from basic theory to modern applications in the field. An excellent example is the chapter on seawater as an electrolytic solution, in which well-developed arguments are used to explain its complex electrolytic nature. The overview that this and some other chapters present is the most significant improvement on the earlier edition—frequently a catalogue of random measurements of chemical species.

The impression that chemical oceanography lags some way behind other fields of science is dispelled by many of the chapters. Others only confirm this impression—for example, the essay on micronutrient elements comprises much work completed twenty years and more ago. The chapter on dissolved organic material in seawater confirmed the unsatisfactory state of research in this area. The all-embracing expression 'dissolved organic matter (DOM)' appeared too often and is of limited usefulness. The inclusion of DDT and PCB seemed out of place, and I was not convinced as to the accuracy or completeness of the information presented on chlorinated hydrocarbons.

The opportunities for criticism in these volumes were few. The books taken as a whole provide a comprehensive picture of chemical oceanography today. To anyone interested in the marine environment I warmly recommend this major work.

M. M. Rhead

obituary

William Marshall Smart, FRSE, FRAS, Regius Professor of Astronomy in the University of Glasgow from 1937–59, died on September 17 at the age of 86.

Professor Smart was born in Scotland and gained his MA at Glasgow in mathematics and natural philosophy in 1910 and his BSc in 1911. He was awarded a scholarship to Trinity College, Cambridge, where he read for the mathematical tripos. After World War I, which he spent in the Royal Navy, he returned to Cambridge as John Couch Adams Astronomer and Lecturer in Mathematics. He was the author of several popular books on astronomy (*The Sun, The Stars and the Universe*, 1928, *Stellar Dynamics*, 1938, and *Some Famous Stars*, 1950) and also published textbooks on the mathematical theory of astronomy. During World War II, he returned to the problems of navigation, publishing

several books which were to play a significant role in the training of navigators in the Navy and Air Force. He was President of the Royal Astronomical Society and a Vice-President of the Royal Society of Edinburgh.

Marshall Kay, Newberry Professor Emeritus of Geology at Columbia University, died on September 3 in New Jersey at the age of 70.

Born in Ontario, Dr Kay gained his first degree in 1924 at the University of Iowa and his PhD at Columbia in 1929. He joined the geology faculty as a lecturer and became assistant professor in 1937, an associate professor in 1941 and a full professor in 1944. In 1967, he became the sixth Newberry Professor of Geology. Dr Kay paved the way for new global tectonics with

his pioneering modern continental drift theory. His theories from reconstructions of continental movements, demonstrating that North America's boundaries were delineated 400 Myr ago when upheavals in the ocean floor consolidated offshore chains of volcanic islands with the mainland, supplanted earlier theories of the origin on the North American continent. Dr Kay suggested that on the basis of his theory of expanding continents, Japan would eventually merge with the Asian mainland. The theory also envisages Alaska again becoming part of Siberia and that the China Sea would 'disappear' into South Asia. "For profoundly influencing the theory and practice of modern stratigraphy", the Geological Society of America presented him with the Penrose Medal. He was also awarded the Kunz Prize by the New York Academy of Sciences.

announcements

Award

The **Gold Medal** of the Canadian Association of Physicists has been awarded to **J. A. Jacobs**.

Appointments

J. L. Links and **J. C. Polkinghorne** have been appointed members of the Science Research Council.

A. J. Buller, **A. P. M. Forrest**, **H. Kay** and **C. M. S. Saunders** have been appointed to the Medical Research Council. **Sir John Gray**, present Secretary to the Council, is to become Deputy Chairman.

International meetings

October 4, **Analytical aspects of ozone and odor control with ozone**, Miami (International Ozone Institute, Skytop Complex, Syracuse University, Merrill Lane, Syracuse, New York 13210, US).

October 14, **Scientific results from the Ariel-5 satellite**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

Person to person

Paris-Reading. Two bedroomed house wanted in or near Paris, convenient for the University of Paris VII, Place Jussieu, for one year from January 1976, possibly in exchange for house in Reading (Dr J. Walker, Physics Department, Reading University, Whiteknights, Reading RG6 2AF, UK; Reading 85123 ext. 388).

Paris-New York. French biochemist, wife and child seek four-room furnished apartment in New York City in exchange for similar accommodation in central Paris for a sabbatical year starting in September 1976 (Dr D. Barritault, 4, rue Française, 75001 Paris, France).

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

October 21–23, **War on waste**, Warwickshire (Exhibition Managements Ltd, 159 Mortlake Road, Kew, Surrey, UK).

November 13–14, **Water structure and transport in biology**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

November 17–20, **Juvenility in woody perennials**, Berlin (Professor A. Karnatz, Institut für Nutzpflanzenforschung Obstbau, T V Berlin, Fachbereich 15 D-1000, Berlin, Dahlen Albrecht Thierweg 3, Germany Fed Rep).

November 19–21, **Nuclear science**, San Francisco (R. S. Larsen, SLAC, Stanford University, PO Box 4349, Stanford, CA 94305).

November 24–26, **Medcomp**, Berlin (AMK, c/o Online Conferences Ltd., Cleveland Road, Uxbridge, UK).

November 29–December 3, **Alcohol and drug dependence**, Bahrain, Arabian Gulf (International Council on Alcohol and Addictions, Box 140, 1001, Lausanne, Switzerland).